THE EFFECT OF BREAKFAST CONSUMPTION ON THE ACUTE RESPONSE OF PLASMA ACYLATED-GHRELIN AND GLUCAGON-LIKE PEPTIDE 1 CONCENTRATIONS IN ADULT WOMEN

by

Thomas A. Hritz, MS, RD, LDN

B.S., University of Pittsburgh, 2003

M.S., University of Pittsburgh, 2007

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This dissertation was presented

by

Thomas A. Hritz, MS, RD, LDN

It was defended on

April 22, 2011

and approved by

Rhobert W. Evans, PhD, Associate Professor, Epidemiology

Diane Helsel, PhD, RD, LDN, CSSD, Assistant Professor, Sports Medicine and Nutrition

Amy D. Rickman, PhD, RD, LDN, Assistant Professor, Health and Physical Activity

Dissertation Advisor: John M. Jakicic, Ph.D., Full Professor, Health and Physical Activity

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Thomas A. Hritz, PhD

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Introduction: A recommended strategy to influence energy balance, which may influence body weight regulation, is to eat breakfast regularly. **Purpose:** The purpose of this study was to examine the impact of breakfast consumption versus a non-breakfast condition on concentrations of the appetite-regulating hormones acylated ghrelin (AG) and glucacon-like peptide 1 (GLP-1), daily energy intake, and subjective ratings of hunger in women. **Methods:** This randomized crossover trial recruited a total of 18 normal weight, overweight, and obese women (age 26.3 ± 6.0 years; BMI 26.8 ± 5.9 kg/m^2). Each participant reported to the research center on two mornings following a minimum 12-hour fast to undergo one of two experimental condtions: breakfast consumption that provided 20% of their estimated daily energy needs, or a waiting period with no breakfast. Study visits were separated by at least 3 days. At each experimental session, participants provided blood samples to measure plasma AG and GLP-1 concentrations and visual analogue scale (VAS) questionnaires to measure subjective hunger and satiety ratings prior to each testing condition (baseline) and at 30, 60, and 120 minutes after each testing condition. Participants also self-reported discretionary intake for the remainder of each testing day in a food and physical activity diary. **Results:** Following breakfast consumption compared to the non-breakfast condition, AG was significantly lower and GLP-1 was significantly higher at the 30-, 60-, and 120-minute time points ($P < 0.001$, each), but there was no difference in total daily energy intake between conditions ($P = 0.199$). In addition, subjective ratings of hunger

significantly correlated with energy intake following the breakfast consumption condition ($P < 0.05$) but not following the non-breakfast condition. Subjective ratings of hunger did not correlate with AG or GLP-1 concentrations. **Conclusion:** Even though a significant acute hormonal response was observed following breakfast consumption when compared to a non-breakfast condition, total daily energy intake between conditions was not significantly different. Thus, further studies are needed to understand the influence of breakfast consumption on energy balance and body weight regulation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Rates of overweight and obesity (body mass index (BMI) = 25.0-29.9 kg/m^2 and ≥ 30.0 kg/m^2, respectively) continue to increase in industrialized countries, especially the United States (U.S.). Ogden et al. reported that the prevalence of overweight in the U.S. in 2003-2004 was 40% in men and 29% in women and the prevalence of obesity was 31% in men and 33% in women [1]. Between 1980 and 2004, the prevalence of obesity in the U.S. increased by 140% in men and by 87% in women [1, 2]. This is important because as obesity rates increase so do rates of related chronic diseases, such as type 2 diabetes mellitus, cardiovascular disease, cerebrovascular disease, and some types of cancer [2, 3]. These diseases are the major cause of morbidity and mortality in the United States today and are the leading cause of 70% of all deaths annually [2, 3]. It is likely that with a reduction in the prevalence of overweight and obesity there will be a concomitant decrease in the prevalence of these chronic diseases.

The increasing availability of inexpensive foods that are energy-dense, high in fat, and low in unrefined carbohydrates coupled with increasingly sedentary lifestyles have followed similar trends with the increasing prevalence of overweight and obesity, particularly in industrialized countries [4, 5]. Weight gain results when energy intake exceeds energy expenditure over a period of time, creating a state of positive energy balance. This occurs when more calories are consumed than are required to fuel metabolic processes and physical activity, resulting in excess energy storage, predominantly in adipose (fat) tissue. To induce weight loss, either energy intake needs to be reduced through limiting calorie consumption or energy expenditure needs to be increased through additional physical activity, creating a state of

negative energy balance. To maintain weight, energy intake must equal energy expenditure, creating a state of energy balance [7-10]. Although past research has examined methods for inducing weight loss and maintaining a healthy weight, rates of overweight and obesity continue to rise [1, 2]. Thus, additional research may be required to understand the physiological and metabolic mechanisms of weight regulation, and also how lifestyle factors such as eating and physical activity behaviors contribute to the paradigm of energy balance.

1.1 CORRELATES OF SUCCESSFUL BODY WEIGHT MAINTANENCE

A variety of lifestyle approaches for weight loss have been examined, and these have typically included variations of diet and physical activity that theoretically affect energy balance. However, only a few components of lifestyle interventions for weight loss have consistently been shown to be associated with long-term weight loss maintenance. The National Weight Control Registry (NWCR) is an observational prospective study to examine successful long-term weight loss and weight maintenance strategies in adults who have lost at least 30 pounds and kept it off for at least one year [6]. Based on data from the NWCR the three strategies most commonly used for maintenance of weight loss among participants are: 1) consuming a low-fat/high-carbohydrate diet, 2) frequent self-monitoring of weight and energy intake, and 3) regular physical activity [6]. More recently data from the NWCR has supported regular breakfast consumption as an important lifestyle behavior for weight loss maintenance [7].

Additional support for the importance of breakfast consumption to improve energy balance and regulation is found in data from several published studies that examined an association between increased breakfast consumption and decreased body weight in normal

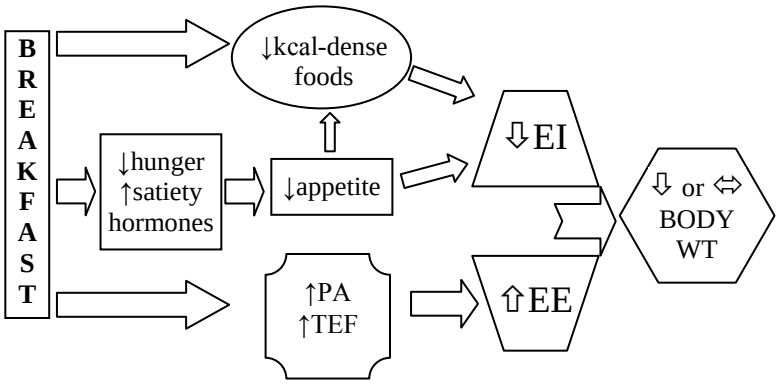
weight, overweight, and obese adults [13-18]. However, other studies have shown the effect of breakfast consumption on body weight may be limited to select population groups or with certain types of foods consumed for breakfast. For example, Song et al. reported an association between breakfast consumption and body weight only in women [8], and Cho et al. found an association between breakfast and lower body weight in those individuals who regularly consumed cereal or quick breads for breakfast, but not in those who regularly consumed meat or eggs for breakfast [8, 9]. Moreover, one additional study failed to find a significant association between daily breakfast consumption and lower body weight in adults [10]. Given these mixed results, further examination of the association between breakfast consumption and factors that may affect body weight appears to be warranted. The first step in this process may be to identify the physiological mechanisms affected by breakfast that may contribute to hunger, satiety, and eating behavior.

1.2 BREAKFAST AND BODY WEIGHT

1.2.1 Pathways Explaining the Impact of Breakfast on Body Weight

Select hypothesized physiological/metabolic pathways by which breakfast may impact body weight are shown in Figure 1. One potential pathway may be that breakfast consumption influences food choices throughout the day and this may influence energy intake and energy balance. Studies supporting this idea have demonstrated that individuals who consume breakfast consume more nutrient-dense foods throughout the day compared to those individuals who do not consume breakfast [11-13]. Nicklas et al., for example, reported the odds of dietary

inadequacy were 2 to 5 times higher in individuals who did not consume breakfast compared to individuals who consume breakfast [13]. Nutrient-dense foods often have fewer calories than foods that are not nutrient-dense. Therefore, it is possible that those who consume breakfast may consume fewer calories throughout the day and have an overall lower daily energy intake.



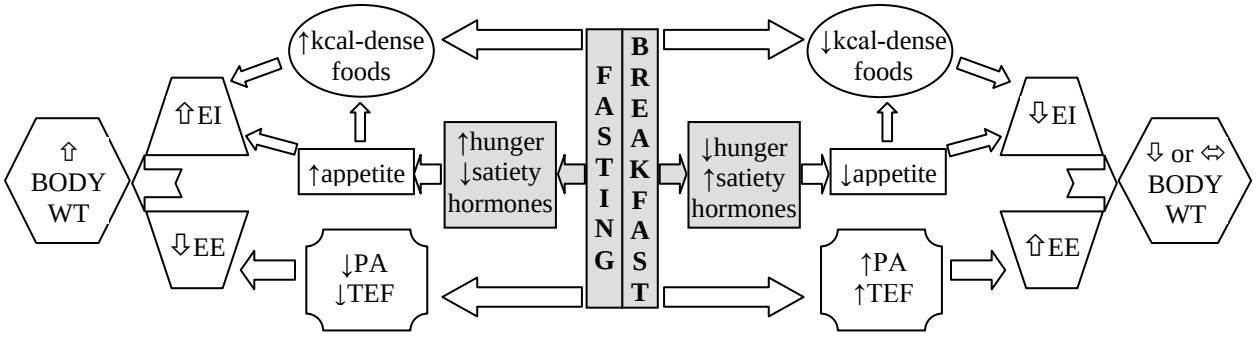
EE=energy expenditure; EI=energy intake; PA=physical activity; TEF=thermic effect of food

Figure 1. Theoretical pathways by which breakfast consumption may impact body weight

A second hypothesis is that breakfast consumption may be associated with a higher level of daily energy expenditure through increased physical activity and an increase in the thermic effect of food (TEF), or the amount of energy expended to digest consumed food, which may influence energy balance and affect body weight. Some studies have reported that individuals who consume breakfast are more physically active in general than individuals who do not consume breakfast [14-16]. Carels et al. reported that exercise duration was significantly longer and total energy expenditure was significantly greater on days when individuals ate breakfast than on days when breakfast was not consumed [15]. Some research has also suggested that increases in TEF could lead to higher energy expenditure throughout the day. Significant

associations have been demonstrated between larger meals [17], more frequent meals [18], or protein-rich meals [19] and greater postprandial energy expenditure, with Johnston et al. showing significantly increased postprandial thermogenesis following a high-protein, low-fat breakfast compared to a high-carbohydrate, low-fat breakfast [19].

It has also been hypothesized that breakfast consumption may stimulate physiological processes that reduce hunger and increase satiety, and this may impact total daily energy intake [20]. Eating breakfast may stimulate hormonal responses, such as decreased levels of ghrelin and increased levels of insulin, leptin, peptide-YY, and glucagon-like peptide 1 (GLP-1). These factors have been shown to decrease hunger and increase satiety, which theoretically may influence total daily energy intake and ultimately influence body weight. Conversely, skipping breakfast may result in a reversal of these hormonal responses, which may result in increased feelings of hunger and decreased satiety, and this may result in an increase in daily energy intake (Figure 2). Farshchi et al. reported that individuals who regularly ate breakfast had a significantly lower total daily energy intake compared to individuals who did not eat breakfast, which may lead to the promotion of weight gain when breakfast is not consumed [21].



EE=energy expenditure; EI=energy intake; PA=physical activity; TEF=thermic effect of

Figure 2. Theoretical pathways by which breakfast consumption and non-breakfast may impact body weight

1.2.2 Appetite Regulating Hormones

This study will focus on the hormonal response to breakfast consumption, as this has been hypothesized as a plausible mechanism to explain the impact of breakfast consumption on body weight [22-52]. However, results of studies examining the influence of breakfast consumption on selected orexigenic peptides (peptides that stimulate appetite) and anorexigenic peptides (peptides that suppress appetite) have been mixed, mostly due to differences in research methodology. Thus, the pathway highlighted in Figure 2 will be investigated in this study. Specifically, this study will focus on the acute effect of breakfast consumption on ghrelin, an orexigenic peptide, and glucagon-like peptide 1 (GLP-1), an anorexigenic peptide. Ghrelin and GLP-1 are of special interest in the hormonal response pathway linking breakfast consumption to body weight, as each has been reported to be particularly responsive to food intake [23, 34].

Ghrelin is a 28-amino acid residue peptide that has been reported to be involved in multiple physiological processes, including the: stimulation of gastric motility and acid secretion;

promotion of adipogenesis; slowing of the metabolic rate; regulation of somatic growth; metabolism of glucose; and the stimulation of growth hormone release [22, 39-41, 53-56]. However, its predominant role is in the stimulation of appetite and food intake [25, 27, 57]. Ghrelin exists in two forms: acylated ghrelin (AG) and des-acyl ghrelin (DG). Research shows that only the acylated form of ghrelin is capable of binding to the growth hormone secretagogue (GHS) receptor in the hypothalamus to stimulate appetite and food intake [35, 55, 56]. Recent studies support this in showing that only ghrelin in its acylated form is significantly associated with increased appetite and decreased energy expenditure, especially during periods of fasting [38-41, 57]. Only two of these studies, though, examined the impact of breakfast on plasma levels of AG [38, 57]. Unfortunately, the scope of these studies was not specific to breakfast and AG and each included fewer than nine participants. Because AG has been reported to play a large role in the stimulation of hunger and initiation of energy intake, and because of a lack of research examining the association between breakfast consumption and AG, additional research is needed to investigate the impact of breakfast consumption on AG.

GLP-1 is noteworthy for its role in mediating hyperglycemia in diabetic individuals through its influence on glucose-dependent insulin secretion [28, 34]. GLP-1 receptor agonists are currently marketed as pharmacological agents that have proven effective in the regulation of glucose homeostasis in individuals with type 2 diabetes mellitus [28, 34]. GLP-1 is also influential in reducing appetite and energy intake in animals and humans [22, 58]. Only two studies have examined the impact of breakfast consumption on GLP-1 [42, 51]. However, the focus of these studies was to examine the effect of the macronutrient composition of meals on GLP-1 and a fasting control condition was not part of the research design. Also, both studies used only young, normal weight participants (mean age 23.3 ± 0.5 and 22 ± 1 y; mean BMI 22.1

± 0.4 and 23.9 ± 0.3 kg/m^2, respectively). Due to the reported role of GLP-1 in the regulation of energy balance and limited research examining the association between breakfast consumption and GLP-1, further investigation is needed to examine the impact of breakfast consumption on GLP-1.

1.3 SPECIFIC AIMS

The primary aims of this study are:

1. To examine the effect of breakfast consumption compared to a non-breakfast condition on the acute response of plasma AG concentrations over a two-hour period.
2. To examine the effect of breakfast consumption compared to a non-breakfast condition on the acute response of plasma GLP-1 concentrations over a two-hour period.
3. To compare the effect of breakfast consumption and a non-breakfast condition on self-reported total daily energy intake.
4. To examine the effect of body mass index (normal weight, overweight, obese) on the acute response of plasma AG and GLP-1 over a two-hour period following a breakfast and non-breakfast condition.

Exploratory aims of this study are:

1. To examine the effect of breakfast consumption and a non-breakfast condition on subjective ratings of hunger and satiety over a two-hour period.

2. To examine the association between subjective ratings of hunger and satiety with plasma AG and GLP-1 concentrations over a two-hour period following breakfast consumption and a non-breakfast period.

1.4 HYPOTHESES

The primary hypotheses of this study are:

1. Plasma AG concentrations will be significantly lower for two hours following breakfast consumption than for two hours following a non-breakfast condition.
2. Plasma GLP-1 concentrations will be significantly higher for two hours following breakfast consumption than for two hours following a non-breakfast condition.
3. Self-reported levels of total daily energy intake will be significantly lower following breakfast consumption than following a non-breakfast condition.
4. Plasma AG levels will be significantly higher and GLP-1 levels will be significantly lower in obese participants than in overweight participants for two hours following breakfast consumption than for two hours following a non-breakfast condition. Plasma AG levels will be significantly higher and GLP-1 levels will be significantly lower in overweight participants than in normal weight participants for two hours following breakfast consumption than for two hours following a non-breakfast condition.

Exploratory hypotheses of this study are:

1. Subjective ratings of hunger will be significantly lower and subjective ratings of satiety will be significantly higher for two hours following breakfast consumption than for two hours following a non-breakfast condition.
2. Subjective ratings of hunger will correlate with plasma AG concentrations and subjective ratings of satiety will correlate with plasma GLP-1 concentrations for two hours following breakfast consumption and a non-breakfast period.

1.5 SIGNIFICANCE

Overweight and obesity are important public health concerns in the United States today, mostly due to the impact they have on chronic disease. Effective strategies to maintain body weight have been reported, but the mechanisms behind their actions have not been thoroughly examined. One recommended strategy for successful weight loss is to eat breakfast regularly. A proposed mechanism to explain the effect of breakfast consumption on body weight is that eating breakfast initiates a hormonal response that improves feelings of hunger and satiety. Improved feelings of hunger and satiety following breakfast may lead to decreased energy intake later in the day which may, in turn, lead to a decrease in body weight over time.

Limited research has been conducted on appetite-regulating hormonal responses following breakfast consumption and results have been mixed. This is due, in part, to differences in research methodology. This study proposes to examine the impact of breakfast consumption on two key appetite-regulating hormones, acylated ghrelin and GLP-1. Acylated ghrelin was chosen to be examined because it is the only known orexigenic gut hormone in humans and has been shown to induce hunger and act as an initiator of energy intake [22, 34]. GLP-1 was chosen

because in addition to increasing satiety and decreasing energy intake, it contributes to the regulation of blood glucose homeostasis and energy balance [28, 34]. It is hypothesized that eating breakfast will stimulate a hormonal response that will improve feelings of hunger and satiety, resulting in a reduction in daily energy intake significant enough to favor weight loss maintenance over time. However, because hunger and satiety are controlled by many factors other than hormones, it is possible that any changes in daily energy intake may not be significant after controlling for the other factors. If a significant, objective relationship between breakfast consumption and AG and GLP-1 levels is demonstrated, it would support the observational data that shows breakfast consumption to be an effective strategy in maintaining body weight as reported in the NWCR.

2.0 REVIEW OF LITERATURE

2.1 INTRODUCTION

Overweight and obesity significantly increase the risk for illness from high blood pressure, high cholesterol, type 2 diabetes, heart disease and stroke, and certain types of cancer [59]. Each of these is directly associated with the leading causes of morbidity and mortality in the United States [2, 3]. Weight loss and maintenance of a healthy body weight, however, reduce these risks for chronic disease [60]. Research has shown that an average weight loss of 22 pounds achieved by dietary interventions was positively associated with significantly lower systolic blood pressure by an average of 7 mm Hg and diastolic blood pressure by an average of 3 mm Hg in overweight hypertensive individuals [60]. Also, weight loss of between 5-13% of body weight was associated with significant improvements in blood cholesterol by as much as –18% for total cholesterol, –22% for LDL-cholesterol, +27% for HDL-cholesterol, and –44% for triglycerides. In addition, weight loss averaging 11 pounds through diet was associated with a 2% decrease in HbA_{1c} over 6 months in overweight diabetic individuals [60]. To reduce the risks for chronic disease, effective strategies are needed to help people lose weight and maintain a healthy body weight. Since individuals are often more successful at maintaining a healthy weight than trying to lose significant amounts of weight and keep it off once obesity is established, these strategies should be initiated as early as possible.

2.2 THE IMPACT OF BREAKFAST ON BODY WEIGHT

Since being established in 1994, the NWCR has followed over 5,000 participants who have maintained at least a 30 pound weight loss for at least 1 year [6]. Wing et al. reported that breakfast consumption was a common behavior among registry participants to maintain weight loss [6]. However, results of other research examining the association between breakfast consumption and body weight have been mixed. There are several studies that support the findings of Wing et al. For example, in a randomized crossover study on normal weight, overweight, and obese women (mean age 29.5 ± 5.9 y, BMI range 23-37 kg/m^2) involving two 6-week trials, Keim et al. demonstrated that when 70% of the participants' daily calories were consumed in two morning meals, significantly more weight was lost than when 70% of daily calories was consumed in two afternoon and evening meals (–3.90 ± 0.19 vs. –3.27 ± 0.26 kg/6 wk, $P < 0.01$) [61]. In a prospective cohort study on men (mean age 57.3 y, mean BMI 25.6 kg/m^2), van der Heijden et al. showed that breakfast consumption was significantly associated with a 13% lower risk of 5 kg weight gain over a 10 year follow-up period, independent of lifestyle and BMI at baseline (multivariate HR = 0.87) [62]. This inverse association was even stronger in men with a baseline BMI ≤ 25 kg/m^2 (multivariate HR = 0.78) [62]. Similarly, in a prospective observational study that monitored adults over one year (mean age 48 y, mean BMI 27.6 kg/m^2), Ma et al. reported that individuals who did not consume breakfast had 4.5 times the risk of obesity as those who regularly consumed breakfast (95% CI 1.57-12.90), and subjects who skipped breakfast at least once during the study had 1.34 times the risk of obesity (95% CI 0.81-2.20) [63]. Finally, in a prospective cohort study on adolescents (mean age 15.9 ± 0.1 y, mean BMI 22.9 ± 0.1 kg/m^2), Niemeier et al. supported the need to begin interventions earlier rather than when obesity is established by reporting that decreased breakfast consumption during

the transition from adolescence to adulthood was significantly associated with increased weight gain in early adulthood ($P < 0.01$) [64].

However, there is additional research that does not support the findings of Wing et al., reporting either no relationship between breakfast consumption and body weight or reporting mixed results. For example, in a cross-sectional parallel group study that examined differences in eating behaviors of normal weight (mean age 49.6 ± 7.2 y, mean BMI 23.8 ± 3.1 kg/m^2) and obese (mean age 47.7 ± 5.9 y, mean BMI 41.0 ± 3.4 kg/m^2) Swedish women, Berteus Forslund et al. found no significant difference in the frequency of breakfast consumption between weight groups (mean ~1.25 vs. ~1.35 meals/person/day at traditional breakfast time, respectively) [10]. In another cross-sectional study on 4,218 adults ≥ 19 years old, Song et al. reported that an association between increased breakfast consumption and BMI < 25 kg/m^2 was not significant in women ($P = 0.1300$) or men ($P = 0.7544$) [8]. However, participants of the study were surveyed only once. In the final model of a retrospective analysis of an observational study, Affenito et al. reported that frequency of breakfast consumption did not predict BMI in pre-adolescent girls as they transitioned to early adulthood ($P = 0.38$) [11]. Finally, mixed results were reported in a cross-sectional study that examined the body weights and breakfast consumption practices of U.S. adults. Cho et al. reported that those who ate ready-to-eat cereal (mean BMI 26.03 kg/m^2), cooked cereal (mean BMI 25.46 kg/m^2), or quick breads (mean BMI 26.16 kg/m^2) weighed significantly less than those who did not consume breakfast (mean BMI 26.92 kg/m^2), but that those who ate meat and eggs for breakfast (mean BMI 27.04 kg/m^2) did not weigh significantly less [9].

The discrepancies in these results are mostly due to differences in research methodology. Most of the reviewed studies that reported an association between increased breakfast

consumption and lower body weight used stronger research designs than those that found weak or no relationships, with Keim et al. [61] using a randomized crossover design and Niemeier et al. [64] and van der Heijden et al. [62] using prospective cohort designs. Studies finding a weak or no association used either cross-sectional [8-10] or retrospective analysis designs [11]. Further research is needed to clarify the methodology examining the association between breakfast consumption and body weight in an effort to better explain why breakfast may impact body weight as reported by Wing et al [7].

2.3 THE IMPACT OF BREAKFAST ON ENERGY INTAKE

One hypothesis to explain how breakfast may impact body weight is that breakfast consumption may stimulate physiological processes that lead to decreased total daily energy intake [20]. Consuming breakfast may blunt the insulin response and maintain it at a constant low level. This in turn may lead to reduced appetite between meals which potentially may lead to decreased energy intake later in the day [20]. The limited research investigating this association in adults, though, has come to differing conclusions. In a randomized crossover trial on lean women (mean age 25.5 ± 5.7 y, mean BMI 23.2 ± 1.6 kg/m^2), Farshchi et al. reported that following two weeks of daily breakfast consumption participants had significantly lower daily energy intake than when following two weeks of skipping breakfast (6.97 ± 0.59 vs. 7.35 ± 0.65 MJ/day, P = 0.001). It was concluded that increased energy intake when skipping breakfast could lead to weight gain [21]. On the contrary, in a cross-sectional study of 504 young adults (mean age 23 y) involving one 24-hour diet recall, Nicklas et al. reported that participants who did not consume breakfast had a significantly lower daily energy intake than those who consumed

breakfast (1990.4 vs. 2558.1 kcals, $P < 0.0001$) [13]. However, differences in lifestyles between those who consumed breakfast and those who did not were not explored. For example, the socioeconomic status and physical activity behaviors of participants were not considered. In another study, Schusdziarra et al. examined food diaries recorded over 10 days by 280 obese participants preparing for a weight loss program (male/female ratio 75/205, mean age 45 ± 0.85 y, mean BMI 36.6 ± 0.2 kg/m^2) and diaries recorded over 14 days of 100 normal weight control participants (male/female ratio 33/67, mean age 42 ± 0.2 y, mean BMI 32.5 ± 0.1 kg/m^2). The researchers reported that obese participants significantly increased total daily energy intake by approximately 400 calories and normal weight participants significantly increased total daily energy intake by approximately 500 calories on days when breakfast was consumed versus days it was not consumed ($P < 0.05$) [65]. Although the well-designed research study by Farshchi et al. found a significant association between increased daily breakfast consumption and decreased total daily energy intake, a physiological pathway to explain the results was not explored [21]. There is a need to further investigate mechanisms that explain how regular breakfast consumption can lead to energy balance and impact body weight. A plausible mechanism to explain the impact of breakfast consumption on body weight involves the response of appetite regulating hormones to breakfast consumption.

2.4 PRIMARY OUTCOME MEASURES

2.4.1 Appetite Regulating Hormones

To regulate energy intake and energy balance, the body produces peptides that stimulate appetite, known as orexigenic peptides, and peptides that suppress appetite, known as anorexigenic peptides. These peptides often interact with one another and modulate feelings of hunger and satiety to control feeding [22, 34]. The main orexigenic peptides associated with increased appetite and energy intake are the hormone ghrelin and the polypeptides neuropeptide Y (NPY) and agouti-related protein (AgRP). Ghrelin has been reported to influence the expression of NPY and AgRP and each responds to food similarly; increasing to the point of meal initiation and then decreasing following meal consumption [22, 34]. The main anorexigenic peptides associated with decreased appetite and energy intake are the hormones insulin, leptin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) [22, 34]. Each has been reported to inhibit food intake, with PYY and leptin inhibiting the action of NPY and AgRP [22, 34]. The gut-based hormones CCK, GLP-1, and PYY work together to reduce food intake over several hours as each is secreted in a different part of the gastrointestinal tract, with CCK being secreted in the duodenum and jejunum, GLP-1 being secreted predominantly in the ileum and colon, and PYY being secreted in the rectum and colon.

The interaction between these peptides is complex, especially since concentrations of some of them have been reported to change with changes in body weight [22, 34]. For example, concentrations of ghrelin, GLP-1, NPY, AgRP, insulin, leptin have been found to be lower in obese individuals, while obesity has no effect on concentrations of PYY and CCK [22, 34]. Of all the orexigenic and anorexigenic peptides, ghrelin and GLP-1 are of special interest in the

hormonal response pathway linking breakfast consumption to body weight, as each has been reported to be particularly responsive to food intake [23, 34]. In fact, it has been suggested that changes in ghrelin and GLP-1 after gastric bypass surgery may contribute to the weight-reducing effect of this procedure [27, 36].

2.4.1.1 Ghrelin

Ghrelin is a 28-amino acid peptide that is mainly produced by the X/A-like endocrine cells in the oxyntic glands of the stomach and upper gastrointestinal tract and, to a smaller degree, in the arcuate nucleus of the hypothalamus [29, 41]. Plasma ghrelin has been observed to correlate with hunger [58]. It increases during periods of fasting, rises further just before spontaneous feeding, and then quickly falls again after eating [22, 54, 58]. Therefore, it has been suggested that ghrelin plays a role in meal initiation [22, 54], and there is data to support this [25, 53, 66]. For example, in an intervention study that followed normal weight and overweight adults (age range 29.1-63.7 y, BMI range 22.0-30.0 kg/m^2) over the course of one day, Cummings et al. demonstrated a mean 78% increase in plasma ghrelin concentrations in the two hours prior to eating each meal and a decrease to baseline levels within one hour after food was consumed [53]. Blom et al. specifically examined plasma ghrelin concentrations with respect to breakfast in a randomized crossover study on normal weight and obese men (mean age 33.2 ± 4.8 and 40.8 ± 4.7 y, mean BMI 23.2 ± 0.5 and 33.2 ± 0.8 kg/m^2, respectively) [25]. The investigators monitored changes in total plasma ghrelin concentrations following a three-day energy restricted and a three-day energy-balanced diet and reported several interesting results. First, following breakfast consumption mean total plasma ghrelin concentrations, averaged across diet type and weight-class, fell to 88.0 ± 5.7% of fasting levels and then returned to 105.8 ± 7.1% of pre-

breakfast fasting levels by the time lunch was requested by participants [25]. Second, the intermeal interval (IMI) between a set breakfast time and a participant-chosen lunch time significantly decreased as the area under the curve (AUC) of the ghrelin response increased (r = –0.42, $P < 0.01$). However, this association was found only in normal weight participants. Finally, lunch preprandial plasma ghrelin concentrations significantly increased as IMI increased ($r = 0.51$, $P < 0.05$). However, this significant correlation only occurred following the three-day energy restricted diet and it was not influenced by BMI. These data demonstrated that plasma ghrelin concentrations increased the longer participants went without eating, and the greater the ghrelin response, the sooner participants started eating, which supports the suggestion that ghrelin plays a role in meal initiation. However, results from the Blom study also suggest that the association may be affected by energy restriction [25]. Unfortunately, a non-breakfast control condition was not part of the study design. Consequently, additional research is needed to investigate the impact of a non-breakfast condition on the association between increased plasma ghrelin concentrations and increased energy intake.

The above studies investigated the association between eating and total plasma ghrelin concentrations [25, 53]. Total plasma ghrelin consists of two major molecular forms, acylated ghrelin (AG) and des-acyl ghrelin (DG). It has been reported that less than 10% of total plasma ghrelin exists as AG [57, 67], with Lucidi et al. reporting that AG accounts for 3-4% of total ghrelin circulating in the blood [57]. Activation of AG by way of acylation of the serine 3 position with an octanoyl group allows it to attach to growth hormone secretagogue (GHS) receptor-1 in the hypothalamus [35, 41, 55, 56, 67]. There it enacts an orexigenic cascade that results in a release of growth hormone, increased appetite, stimulation of food intake, and a decrease in energy expenditure, especially during fasting [41, 54, 68]. Des-acyl ghrelin is not

capable of binding to the GHS receptor and, therefore, does not initiate the orexigenic effect of AG [35, 41, 55, 56, 67].

Acylated-ghrelin is the only peripheral signal hormone that increases food intake [68]. Studies have demonstrated the effects of AG on energy intake when administered either subcutaneously or intravenously. In a randomized controlled trial on normal weight adults (mean age 25.4 ± 2.9 y, mean BMI 22.5 ± 1.5 kg/m^2), Druce et al. reported significantly greater energy intake and perceived palatability of food at breakfast following subcutaneous administration of AG prior to breakfast when compared to saline administration (5076 ± 691 vs. 4230 ± 607 kJ, $P = 0.04$, and 81.1 ± 3.6 vs. 70.0 ± 4.4 mm on VAS scale, $P = 0.03$, respectively) [69]. Similarly, in a randomized crossover trial on normal weight and overweight adults (mean age 25 ± 1.1 y, mean BMI range 19.8-26.8 kg/m^2), Wren et al. reported significantly greater energy intake and subjective hunger ratings at lunch following intravenous infusion of AG prior to and after breakfast when compared to saline infusion (5997 ± 413 vs. 4713 ± 344 kJ, respectively, $P < 0.001$ and 16 ± 10% greater increase in hunger ratings at 120 min. and 46 ± 20% greater increase at 240 min. after breakfast, $P < 0.05$) [70]. These studies, though, did not examine the association between endogenous AG and energy intake [68-70].

Only two studies have specifically examined the effect of breakfast consumption and fasting on endogenous plasma AG concentrations, but neither was appropriately powered [38, 57]. In a randomized crossover study involving eight young, normal weight and mildly overweight men (mean age 24.5 ± 3.7 y, mean BMI 24 ± 2.1 kg/m^2) comparing the temporal profile of AG concentrations over 26.5 hours following consumption of a standard mixed breakfast and a non-breakfast condition, Liu et al. reported that AG concentrations quickly decreased to nadir levels within one hour post-breakfast and then increased to exceed breakfast

preprandial levels within two additional hours. This was similar to the total plasma ghrelin response following breakfast consumption as previously discussed [38]. In a similar randomized crossover study that monitored AG concentrations for four hours following a breakfast consumption condition and non-breakfast condition in six normal weight adults (mean age 36 ± 2 y, mean BMI 23 ± 0.7 kg/m^2), Lucidi et al. [57] reported a pattern similar to the study by Liu et al. [38] following consumption of a standard mixed breakfast. However, results of the two studies differed regarding the non-breakfast condition. Liu et al. [38] reported that during four hours of monitoring, plasma AG concentrations stayed fairly flat, at a level similar to nadir levels in the fed condition. Lucidi et al. [57] reported that during four hours of monitoring, plasma AG concentrations, though fairly flat, were significantly higher in the non-breakfast condition compared to the breakfast consumption condition ($P < 0.01$). However, in the study by Liu et al. [38] AG monitoring during the fasting condition began after the participants had been fasting for 37.5 hours of a 61.5 hour fast, while in the study by Lucidi et al. [57], monitoring began after an overnight fast. Due to a limited number of studies, with differing results and methodologies, that examined the association between breakfast consumption and endogenous AG concentrations, additional research is needed to explore this relationship.

Des-acyl ghrelin accounts for over 90% of total plasma ghrelin [57, 67], and until recently it was believed to be biologically inert. However, research shows that certain actions of DG inhibit actions of AG. For instance, in a randomized controlled trial on mice that monitored energy balance following intracerebroventricular (ICV) injection of AG, DG, or artificial cerebrospinal fluid, Asakawa et al. reported that DG had a significant inhibitory effect on energy intake ($P < 0.01$) and gastric emptying rate ($P < 0.05$) over a two hour period [71]. Also, transgenically created mice designed to overexpress DG gained less weight with reduced fat

mass compared to unaltered mice (33.90 ± 0.631 vs. 37.69 ± 1.673 g at 44 weeks, $P < 0.05$) [71]. Results of another randomized controlled animal trial showed that DG significantly inhibited energy intake in goldfish over a 60 minute period after ICV injection of AG, DG, or saline ($P < 0.05$ at 15 and 60 min., $P < 0.01$ at 30 and 45 min.) [40]. It is possible that the relationship between increased DG concentrations and decreased energy intake is the result of the effect DG has on the insulin response. As evidence, Qader et al. reported that DG compromises the appetite regulating effect that AG has on the secretion of pancreatic hormones. In comparing the effects of AG alone or in combination with DG, Qader et al. reported that the presence of DG ameliorated the effect of AG on the secretion of insulin, pancreatic polypeptide, somatistatin and glucagon ($P < 0.001$) [72]. Due to the impact that DG may have on the appetite-regulating actions of AG, and due to the limited research examining the response of AG to breakfast consumption, additional investigation of the relationship is warranted to explain the impact of breakfast consumption on body weight.

The appetite-regulating effects of AG and DG may be impacted by fasting. Research has shown that the ratio of DG to AG may change with long-term fasting [38, 56]. In a randomized controlled trial on rats, Toshinai et al. reported that the DG to AG ratio was markedly increased following a 42 hour fast (1.3:1 in fed rats vs. 3:1 in fasted rats) while the total plasma ghrelin level did not change [56]. Similarly, in a randomized crossover trial on young, normal weight and overweight males (mean age 24.5 ± 3.7 y, mean BMI 24 ± 2.1 kg/m^2), Liu et al. showed a significant increase in the DG to AG ratio following a 61.5 hour fast, with an increase in DG by 19% (102.2 ± 13.4 vs. 121.9 ± 11.8 pg/ml, $P = 0.040$) and a decrease in AG by 58% (27.9 ± 3.9 vs. 11.8 ± 1.7 pg/ml, $P = 0.0025$) while total plasma ghrelin did not change (130.1 ± 13.7 vs. 133.7 ± 12.5 pg/ml, $P = 0.66$) [38]. However, the impact of fasting on the DG to AG ratio may

only be evident following long-term fasting. In a randomized crossover trial on normal weight adults (mean age 36 ± 2 y, mean BMI 23 ± 0.7 kg/m^2), Lucidi et al. found the DG to AG ratio did not change following an overnight fast, with AG accounting for similar amounts of total plasma ghrelin following both breakfast consumption and non-breakfast conditions (range 3.4-3.9% vs. 2.8-3.0%, respectively, *P* = NS) [57]. Due to the reported effects of, 1) fasting on changes in the ratio of DG to AG, and 2) DG on inhibition of the appetite-regulating actions of AG, additional research is needed to further explore the impact of breakfast consumption and a non-breakfast condition on plasma AG concentrations.

2.4.1.2 Glucagon-Like Peptide 1

Unlike ghrelin, which is the only known appetite-stimulating hormone found in the gut, GLP-1 is one of many gut peptides associated with appetite suppression and regulation of satiety. The sensation of satiety is dependent upon a cascade of physiological responses, in which the gut hormones CCK, GLP-1, and PYY play a primary role [54, 73]. Cholecystokinin is produced in the duodenum and upper jejunum and is released quickly into circulation after ingestion of food, peaking within 30 minutes of meal initiation. Its main role is to induce meal termination [34, 54, 74]. GLP-1 and PYY are produced and released by the L-cells of the distal gut, ileum, colon, and rectum in response to intestinal nutrients [34, 58, 73, 74]. Levels of circulating GLP-1 peak approximately 60 minutes after meal initiation [73] and levels of PYY peak in the second hour after meal initiation [34]. Therefore, it is believed that GLP-1 and PYY do not play a significant role in meal termination but instead impact intermeal satiety, leading to decreased energy intake at subsequent meals [73]. This occurs through several actions. For instance, GLP-1 and PYY have been shown to slow gastric emptying [52, 75-77], leading Blundell et al. to conclude that prolonged gastric extension would lead to release of other satiety-related gastrointestinal

hormones and extended vagal stimulation of receptors involved in the control of food intake [73]. In addition to slowing gastric emptying, GLP-1 has been reported to reduce gastric acid secretion in humans and elicit symptoms of visceral illness in rats, including conditioned taste aversion, all of which contribute to feelings of satiety between meals [34, 46, 58, 73, 74, 78]. However, the main reason GLP-1 is unique as a component of the satiety cascade relates to its role in glucose homeostasis.

GLP-1 is classified as an incretin, meaning it stimulates insulin secretion from the pancreas [28, 73, 79]. It also inhibits glucagon secretion [79, 80]. In addition to being released from the distal gut, GLP-1 is also expressed by the alpha cells of the pancreas [81]. As evidence of the role of GLP-1 in glucose homeostasis, Edwards et al. reported a significant increase in postprandial hyperglycemia following IV infusion of exendin 9-39, a known antagonist of GLP-1 receptors, compared to infusion of saline (plasma glucose concentrations 8.67 ± 0.35 vs. 7.67 ± 0.35 mmol/L, respectively, $P < 0.005$) in a randomized crossover trial on young, normal weight adults (mean age 25.5 ± 0.9 y, mean BMI 23 ± 1 kg/m^2) [82]. The effect of GLP-1 on glucose metabolism coupled with the previously reviewed effect of DG on the secretion of pancreatic hormones [72] could help explain the association between regular breakfast consumption and decreased energy intake. The effects that GLP-1 has on increased satiety and glucose homeostasis may lead to decreased energy intake at subsequent meals.

Three randomized crossover trials examined the effects of IV infusions of GLP-1 versus a placebo on energy intake, appetite, and glucose homeostasis [77, 79, 83]. In all three, energy intake was significantly lower following GLP-1 infusion compared to the placebo. Naslund et al. [77] reported a mean 21 ± 6% decrease in energy intake in a study on eight obese men (mean age 35.0 ± 3.8 y, mean BMI 45.5 ± 2.3 kg/m^2) (907.6 vs. 1,027.0 kcals at lunch and 549.3 vs. 740.4

kcals at dinner, respectively, $P = 0.05$ for both), while Flint et al. [79] reported a mean 12% decrease in a study on 20 young, normal weight men (mean age 25.5 y, mean BMI 23 kg/m^2) (883.7 ± 72 vs. 1,003.2 ± 48 kcals, $P = 0.002$). Gutzwiler et al. reported a dose-dependent relationship between increased IV infusions of GLP-1 and decreased energy intake in a study on 16 young, normal weight men (mean age 23.6 ± 0.5 y, mean BMI not reported) using doses of 0.375, 0.75, and 1.5 pmol/kg/min (1520 ± 95, 1451 ± 101, and 1107 ± 84, respectively, vs. 1627 ± 97 kcals, $P < 0.001$ overall). However, differences in energy intake between the GLP-1 condition and placebo condition were significant only at the 1.5 dose and the 0.75 dose of GLP-1 ($P < 0.001$ and < 0.05, respectively) [83].

With respect to subjective ratings of appetite, results were also similar. In all three studies, ratings of hunger were significantly lower and ratings of satiety were significantly higher following infusion of GLP-1 compared to the placebo. Naslund et al. reported significantly smaller increases in ratings of hunger and prospective consumption and a significant decrease in ratings of fullness between breakfast and lunch ($P < 0.05$ for each) [77]. Also, between lunch and dinner there were significantly smaller increases in ratings of hunger ($P = 0.01$) and desire to eat ($P < 0.05$) [77]. Flint et al. [79] and Gutzwiler et al. [83] reported significantly greater ratings of fullness ($P = 0.028$ and $P < 0.01$, respectively) and significantly lower ratings of hunger ($P = 0.012$ and $P < 0.05$, respectively). Flint et al. also reported significantly higher ratings of satiety ($P = 0.013$) and significantly lower ratings of prospective food consumption ($P = 0.012$). However, in the Gutzwiler study, differences were only significant following the highest GLP-1 dose. This association between increased plasma GLP-1 concentrations and reduced hunger and increased satiety may be evidence of the fact that gastric emptying was significantly delayed by 50% ($P <0.001$) after breakfast and lunch following infusion of GLP-1

compared with placebo as reported by Naslund et al. [77]. Finally, Naslund [77] and Flint [79] also examined changes in ratings of palatability of the test meals and neither found significant differences between testing conditions, challenging research that demonstrated symptoms of conditioned taste aversion and visceral illness in rats following infusion of GLP-1 [46, 78].

Although postprandial hyperglycemia was attenuated following GLP-1 infusion compared to placebo infusion in all three studies [77, 79, 83], differences were significant in only two of them. Naslund [77] and Flint [79] reported significantly lower plasma glucose concentrations following infusion of GLP-1 compared to placebo after all meals ($P < 0.001$ and 0.0001, respectively), but in both studies plasma insulin concentrations were significantly lower following GLP-1 infusion after breakfast only ($P < 0.001$ and 0.002, respectively). Flint et al. also examined glucagon levels and reported that serum concentrations were significantly lower following GLP-1 infusion compared to placebo after breakfast only ($P < 0.001$) [79]. The possible reasons for the decrease in insulin and glucagon concentrations following breakfast only were not discussed by the investigators. Lastly, Gutzwiler et al. reported that although the insulin response dose dependently increased following infusion of GLP-1, the differences compared to placebo infusion were not significant. This could have occurred because glucose was used as the control placebo [83].

Verdich et al. conducted a meta-analysis of nine studies examining the effect of IV infusion of GLP-1 versus a placebo on energy intake, appetite, and glucose homeostasis in 147 participants [52]. The investigators reported a mean reduction in total energy intake of 173.1 kcals ($P < 0.001$) or 11.7% ($P < 0.001$) following IV infusion of GLP-1 compared to a placebo. The reduction in energy intake was higher in normal weight participants than overweight participants, but the difference was not significant (205.5 vs. 132.4 kcals and 13.2 vs. 10.5%,

respectively). Also, the reduction in total energy intake significantly correlated with the increasing GLP-1 infusion rate ($r = 0.40$, $P < 0.001$) and was similar between weight classes, suggesting a dose-dependent relationship between GLP-1 concentrations and subsequent energy intake that is not impacted by BMI. Verdich et al. also reported that differences in plasma GLP-1 concentrations significantly correlated with differences in ratings of fullness ($r = –0.38$, $P = 0.013$) and prospective consumption ($r = 0.40$, $P = 0.008$), but not with hunger ($r = 0.26$, $P = 0.09$). However, differences in subjective ratings of satiety and hunger did not significantly correlate with the reduction in energy intake. The investigators also reported that a reduction in gastric emptying rate significantly correlated with increases in GLP-1 concentrations but not placebo ($P < 0.01$). However, the three studies of the meta-analysis examining gastric emptying rate involved only overweight participants. Finally, there were no significant differences in ratings of visceral illness following IV infusion of GLP-1 when compared with placebo, further challenging the research that demonstrated symptoms of conditioned taste aversion and visceral illness in rats following infusion of GLP-1 [46, 78].

Since each of these studies involved exogenous infusions of GLP-1 [52, 77, 79, 83], it is difficult to conclude that similar responses in energy intake, ratings of hunger and satiety, and glucose homeostasis would be realized following increases in normal endogenous concentrations of GLP-1. To complicate comparisons further, these studies used supraphysiological doses of GLP-1 to elicit responses in participants. As seen in the dose-dependent study by Gutzwiler et al., significant results were only realized following infusion of supraphysiological doses of 0.75 and 1.5 pmol/kg/min. Results following dose levels of GLP-1 that reflected normal endogenous concentrations (0.375 pmol/kg/min) were not significant [83]. Another weakness noted is that all of the individual studies included only men as participants and none were statistically

powered, including only between 8 and 20 men [77, 79, 83]. However, the meta-analysis study by Verdich et al. pooled data from 147 participants for its analysis and reported similar results [52]. Unfortunately, all of the participants were men also, making it difficult to generalize results. Together these studies demonstrate the physiological effect that GLP-1 has on energy intake, appetite, and glucose homeostasis. The significant results support the need for additional research to examine the response of endogenous GLP-1 to breakfast consumption in an effort to explain the impact of breakfast consumption on body weight.

Only one research study specifically examined the effect of breakfast consumption on endogenous plasma GLP-1 concentrations [84]. However, this between-subjects study was designed to examine differences in outcomes in obese male participants before and after weight loss (mean BMI before 38.7 and after 33.0 kg/m^2) as compared with normal weight controls (mean BMI 23.1 kg/m^2). Also, a non-breakfast control condition was not part of the study design, so the effect of breakfast consumption compared to a non-breakfast condition on the acute response of endogenous plasma GLP-1 concentrations can not be assessed. In the study, Verdich et al. reported no correlation between plasma GLP-1 concentrations and subsequent energy intake or gastric emptying rate measured scintigraphically in either population, and only a weak correlation was seen between GLP-1 concentrations and subjective appetite ratings (r^2 = 0.25, $P < 0.03$ in obese subjects, $r^2 = 0.43$, $P = 0.02$ in normal weight subjects) [84]. However, the study did report an inverse correlation between decreased AUC of GLP-1 and increased AUC of insulin in obese subjects ($r^2 = 0.31$, $P < 0.02$), which is contrary to the response reported in the previously reviewed studies [77, 79, 83]. The investigators felt that the inverse correlation was the result of the obese state of the participants in which fasting plasma insulin concentrations are often increased and plasma GLP-1 concentrations are decreased when compared to normal

weight individuals. The impact of BMI on pre- and postprandial plasma GLP-1 concentrations is reviewed below. The results of this between-subjects study helped to shed light on the response of GLP-1 to breakfast consumption. However, additional investigation of within-subject differences following breakfast consumption and non-breakfast conditions is needed to clarify why breakfast consumption may have an impact on body weight.

Although they did not specifically examine the effect of breakfast consumption on endogenous plasma GLP-1 concentrations, several other studies included postprandial changes in GLP-1 concentrations as part of the outcome measures [26, 37, 42, 85, 86]. In addition, subjective appetite ratings were measured in two studies [26, 42] and postprandial changes in plasma insulin and glucose concentrations were monitored in three studies [26, 85, 86]. As with the study by Verdich et al [84], a non-breakfast control condition was not part of the research design in any of the studies, so within-subject differences in the response of endogenous GLP-1 to breakfast consumption versus a non-breakfast condition can not be fully assessed. Results demonstrating the postprandial temporal pattern of the GLP-1 response to breakfast consumption differed among the studies. In three studies, plasma GLP-1 concentrations peaked at approximately 30 minutes following breakfast consumption and trended toward baseline levels within 120 minutes [37, 85, 86]. However, in two other studies, plasma GLP-1 concentrations peaked at approximately 60 minutes or later and trended back toward baseline levels after 120 minutes following consumption of high-protein or high-carbohydrate dairy breakfasts [26] or breakfasts rich in protein, carbohydrate, or fat [42]. The aim of each of these studies, though, was to examine the impact of different macronutrients on the GLP-1 response. It is likely that the differences in temporal patterns of the GLP-1 response are the result of the macronutrient composition of the test meal used. The impact of the macronutrient content of foods on

postprandial plasma GLP-1 concentrations is reviewed below. The only outcome in the above studies to be correlated with endogenous plasma GLP-1 concentrations was subjective appetite ratings. Adam et al. reported that subjective ratings of satiety significantly increased in relation to increased concentrations of GLP-1 at 60 minutes ($r = 0.42$, $P = 0.02$) and 90 minutes ($r = 0.39$, $P = 0.03$) following breakfast consumption [85]. This is in agreement with the research reviewed above that showed an association between exogenously infused GLP-1 and decreased appetite [77, 79, 83]. Finally, even though postprandial changes in plasma insulin and glucose concentrations were not correlated with changes in GLP-1 concentrations, a comparison of temporal trends of the hormones following breakfast consumption supports the following research studies reporting a positive association between GLP-1 and the insulin response. In two studies [26, 42], glucose levels peaked and returned to preprandial baseline levels by the time plasma GLP-1 concentrations peaked, while in another study [86] glucose levels remained flat during the entire course of monitoring even though plasma GLP-1 concentrations peaked at 60 minutes. This may have been due to the use of a high-protein gelatin test meal that was artificially sweetened [86]. In all three studies, insulin levels peaked approximately 30 minutes prior to the peak of plasma GLP-1 concentrations and then trended to baseline levels at rates similar to GLP-1 [26, 42, 86]. These trends support research demonstrating an association between increased plasma GLP-1 concentrations and glucose homeostasis that was reviewed above. An examination of the response of plasma GLP-1 concentrations to breakfast consumption is needed due to the lack of research investigating differences in the response of GLP-1 between breakfast consumption and non-breakfast conditions. A better understanding of this association may help to explain the relationship between breakfast consumption and body weight.

In reviewing the existing literature, there is evidence to support the hypothesis of a response in plasma AG and GLP-1 concentrations to breakfast consumption as a plausible mechanism to explain the impact of breakfast consumption on body weight. However, because the results of the literature have been mixed, mostly due to differences in research methodology, this study will examine the acute effect of breakfast consumption on plasma AG and GLP-1 as part of the pathway that links breakfast consumption to a healthy body weight.

2.5 BREAKFAST MEAL CONSIDERATIONS

2.5.1 Defining Breakfast

An important factor in the discrepancies in results of research investigating the association between breakfast consumption and body weight is differences in how each research team defined the variable breakfast. In a paper that reviewed the impact of breakfast consumption on weight loss, Ruxton and Kirk concluded that conflicting results arose from lack of a standard definition of breakfast [87]. Due to the fact that breakfast consumption and a non-breakfast condition are independent variables in the proposed study, a clear definition of breakfast is important to ensure that outcome measures are comparable between study conditions. Of the research studies reviewed in sections 2.2, 2.3, and 2.4, only three specifically defined the variable breakfast [8, 11, 88]. Song et al. allowed the participants to identify breakfast in self-reporting their daily intake [8]. The other two studies, though, provided more objective definitions. Affenito et al. considered breakfast as any eating between 5:00 a.m. and 10:00 a.m. on weekdays and between 5:00 a.m. and 11:00 a.m. on weekends [11], and Siega-Riz et al.

considered breakfast as any food or beverage consumed between the hours of 5:00 a.m. and 10:00 a.m. [88].

Of all the research studies reviewed for this paper, only five others defined breakfast. Like Song et al., two allowed the participants to identify what breakfast was [9, 15]. A study by Haines et al. used guidelines similar to those of Affenito [11] and Siega-Riz [88] in considering breakfast as any consumption between 5:00 a.m. and 9:00 a.m. [12]. Nicklas et al. did not take the timing or frequency of meal consumption into consideration and instead defined breakfast as food or a mixture of foods that were at least equal in macronutrient values to that of one serving of milk [13]. Also, in considering studies for a review article, Timlin et al. accepted those that defined breakfast as the first meal of the day that was consumed within two hours of waking or before the start of daily activities and typically no later than 10:00 a.m. and was of a calorie level between 20% and 35% of total daily energy needs [20]. In the proposed study, the definition of breakfast will follow criteria used in the studies by Haines et al. [12], Affenito et al. [11], and Siega-Riz et al. [88] since their criteria were based on results from the Nationwide Food Consumption Survey and the Continuing Survey of Food Intakes by Individuals, two large national surveys established by the U.S. Department of Agriculture (USDA). The definition will also include criteria used in the study by Nicklas et al. since it considered a minimum caloric content [13]. In the proposed study, breakfast is defined as consumption of a food or mixture of foods that equals or exceeds the kilocalorie content of one serving of milk (approximately 120 kilocalories) between the hours of 5:00 a.m. and 10:00 a.m. on weekdays and between 5:00 a.m. and 11:00 a.m. on weekends [11-13, 88].

2.5.2 Macronutrient Composition of the Breakfast Meal

Another factor that may have contributed to the discrepancies in results of research investigating the association between breakfast consumption and body weight is differences in the macronutrient content of the breakfast meal used in each study. The macronutrient content of the breakfast meal needs to be considered as it may mediate a relationship between breakfast consumption and plasma AG and GLP-1 concentrations, ratings of hunger and satiety, or energy intake. Three macronutrients provide nearly all of the energy for the typical person: carbohydrate, fat, and protein [89]. Carbohydrate and protein provide 4 kilocalories per gram while fat provides 9 kilocalories per gram [89]. Based on the USDA Food Guide from the *Dietary Guidelines for Americans, 2005*, it is recommended that a healthy diet is comprised of approximately 55% carbohydrate, 30% fat, and 15% protein [2].

Previous research has demonstrated that each of these macronutrients may impact plasma AG and GLP-1 concentrations, ratings of hunger and satiety, and subsequent energy intake differently. However, results have been mixed. Johannes Erdmann and colleagues have investigated the impact of macronutrients on plasma ghrelin concentrations, ratings of hunger and satiety, and subsequent energy intake following breakfast through several randomized crossover trials on normal weight and obese adults [30-33]. Erdmann and his research associates came to several conclusions with respect to carbohydrate. First, total plasma ghrelin concentrations significantly decreased following carbohydrate-rich meals for up to four hours, with the exception of potatoes, after which total ghrelin concentrations significantly increased [30-33]. Second, even though total plasma ghrelin concentrations significantly decreased following most carbohydrate meals, feelings of hunger and satiety did not always correlate to levels of total plasma ghrelin [30, 31, 33]. Third, in one study subsequent energy intake

following carbohydrate-rich foods (790 ± 77 kcals) was significantly greater than subsequent energy intake following protein- and fat-rich meals (721 ± 79 and 613 ± 75 kcals, respectively). One exception pertained to fruit and vegetable sources of carbohydrate which resulted in subsequent energy intake (988 ± 86 and 972 ± 85 kcals, respectively) greater than that of the carbohydrate-rich foods [33]. However, in another study comparing carbohydrate- and protein-rich foods, subsequent energy intake was not significantly different following consumption of each meal (597 ± 39.6 and 621 ± 44.5 kcals, respectively) [31].

With regard to fat, Erdmann et al. found that total plasma ghrelin concentrations remained flat and then significantly decreased following a fat-rich meal in one study (nadir 415 ± 45.2 pg/ml at 180 min.) [32], while in another study ghrelin significantly increased (peak 509 ± 77 pg/ml at 45 min.) [33]. Erdmann et al. observed ratings of hunger and satiety and subsequent energy intake following a fat-rich meal in only one study and found no significant correlation between total plasma ghrelin concentrations and hunger and satiety ratings, but did find that subsequent energy intake following a fat-rich meal (613 ±75 kcals) was significantly less than subsequent energy intake following meals rich in protein, carbohydrate, fruit, and vegetables (721 ± 79, 790 ± 77, 988 ± 86, and 972 ± 85 kcals, respectively) [33].

Finally, in investigating outcomes following meals rich in protein, Erdmann et al. came to several additional conclusions. First, total plasma ghrelin concentrations significantly increased following protein-rich meals for as much as four hours [31-33]. Second, feelings of hunger and satiety did not correlate to levels of total plasma ghrelin and were similar to those following carbohydrate-rich meals [31, 33]. Third, as above, subsequent energy intake following a protein-rich meal was significantly greater than following a fat-rich meal [33], and either similar to or significantly less than following a carbohydrate-rich meal [31, 33].

Summarizing the outcomes from Erdmann's research, it appears that, with exceptions, total plasma ghrelin concentrations generally decrease following consumption of carbohydrate-rich foods and increase following consumption of protein-rich foods, ratings of hunger and satiety do not correlate well with total plasma ghrelin levels among all of the macronutrients, and subsequent energy intake varies following consumption of each of the macronutrients. It should be noted that Erdmann examined changes in total ghrelin and not AG which could explain the lack of correlation between levels of plasma ghrelin and ratings of hunger and satiety.

Other research reported different results. In a non-randomized crossover study on young, normal weight men (mean age 20.5 ± 2.5 y, mean BMI 21.6 ± 1.9 kg/m^2), Blom et al. reported that although the total AUC of the ghrelin response was a significant ~45% greater following a protein-rich breakfast than following a carbohydrate-rich breakfast (P <0.01), the total AUC of the AG response was not significantly different. They also found the AUC of GLP-1 to be ~66% greater following the protein-rich breakfast than following the carbohydrate-rich breakfast, but the difference was not significant (P = 0.10). In addition, subjective ratings of hunger and satiety did not correlate with AG or GLP-1 concentrations and no significant associations were found between the AUC of the ghrelin response and energy intake at lunch. Examining an association between the AUC of GLP-1 and subsequent energy intake was not within the scope of the study [26]. In another randomized crossover adult study (mean age 55.3 ± 2.9 y, mean BMI 29.2 ± 2.2 kg/m^2), Greenman et al. reported that total plasma ghrelin levels significantly decreased following a high glucose breakfast (P < 0.0001), significantly decreased following a high fat breakfast, but only in women (P = 0.029), and had no significant change following a high protein breakfast. Finally, in another randomized crossover study on young, normal weight adults (mean age 23.3 ± 0.5 y, mean BMI 22.1 ± 0.4 kg/m^2), Raben et al. found no significant differences in

the AUC of GLP-1, in subjective ratings of hunger and satiety, or in subsequent energy intake following protein-rich, carbohydrate-rich, and fat-rich breakfasts [42]. Summarizing these studies, plasma AG concentrations responded similarly following carbohydrate consumption and protein consumption even though total plasma concentrations differed, GLP-1 concentrations trended higher following protein consumption in one study but not in another, and subjective ratings of hunger and satiety and subsequent energy intake were similar following consumption of carbohydrate, protein, and fat.

Based on the equivocal results of the above research studies, the macronutrient composition of the breakfast meal needs to be considered in the proposed study as it may impact stimulation or inhibition of AG or GLP-1, subjective ratings of hunger and satiety, and subsequent energy intake. To control for the macronutrient content of the breakfast meal, a mixed meal will be used and comply with the macronutrient profile recommendations for a healthy diet suggested by the USDA Food Guide of the *Dietary Guidelines for Americans, 2005* [2]. The meal will consist of approximately 45-55% carbohydrate, 30-35% fat, and 15-20% protein.

2.5.3 Energy Content of the Breakfast Meal

One last factor that may have contributed to the discrepancies in results of research investigating the association between breakfast consumption and body weight is differences in the energy content of the breakfast meal used in each study. The energy content of the breakfast meal has been shown to impact plasma ghrelin concentrations proportionally. In a three-stage randomized crossover trial on normal weight adults (mean age 22.5 y, mean BMI 22.6 kg/m^2), Callahan et al. demonstrated significantly increasing plasma ghrelin responses to breakfast meals that provided

7.5%, 16%, and 33% of the participants' estimated daily energy expenditure (nadir levels 80.2 ± 2.8, 72.7 ± 2.7, and 60.8 ± 2.7% of baseline, respectively, $P < 0.001$) [90]. Therefore, in the proposed study it will be important to provide a breakfast meal that contributes a standardized percentage of the estimated daily energy expenditure of participants to control for the effect of the energy content of the meal on the ghrelin response.

Cross-sectional studies, intervention studies examining free-living conditions, and retrospective analysis studies have demonstrated that breakfast contributes approximately 10% to 22% of the total daily energy intake of U.S. adults, with an average range of approximately 15% to 20% [8, 12, 13, 15, 51]. In an intervention study examining eating patterns following a behavioral weight loss program, Carels et al. reported that breakfast contributed 14.6% of total daily energy intake in overweight adults [15]. Also, in a retrospective analysis of U.S. adults who were NHANES 1999-2000 respondents, Song et al. reported that 9.9% to 18.6% of total daily energy came from breakfast [8]. Finally, in a review paper Ruxton and Kirk reported that breakfast contributed from 6% to 20% of total daily energy intake in children worldwide and that the contribution of breakfast to total energy intake in adults was often lower than or similar to that of children. However, the paper did not give a specific range for adults [87]. Based on these sources, breakfast contributes approximately 14% of total daily energy intake in adults.

Other sources, though, reported a larger contribution from breakfast. Haines et al. reported in a trends analysis pool of three cross-sectional studies on breakfast consumption by U.S. adults that breakfast contributed from 18% to 22% of total daily energy intake [12]. Similarly, Nicklas et al. reported a 19% contribution [13], and Siega-Riz et al. reported a less than 25% contribution in U.S. adults in their cross-sectional studies [88]

. Finally, in a Dutch randomized controlled trial investigating the effects of a high and normal soy protein breakfast on satiety hormones, ratings of satiety, and energy intake, Veldhorst et al. used a breakfast meal for an intervention that contained 20% of daily energy requirements because it was the average reported for the general population of the Netherlands [51]. The mean contribution of breakfast based on these results is approximately 21%. Based on the above research and to ensure adequate intake for statistical comparisons, the proposed study will provide participants with a breakfast meal that will account for 20% of their estimated daily caloric needs.

2.6 ADDITIONAL FACTORS THAT IMPACT APPETITE REGULATING HORMONES

2.6.1 Age

There is some research that demonstrates changes in fasting and postprandial ghrelin levels with age. In a pre-test, post-test intervention study that compared fasting and postprandial ghrelin levels in older and younger adults (mean age 75.2 ± 1.8, BMI 21.1-28.3 kg/m^2 vs. 28.1 ± 0.7 y, BMI 18.9-24.5 kg/m^2), Di Francesco et al. reported that fasting AG levels were significantly lower in elderly participants (42.5 ± 4.8 vs. 62.8 ± 9.5 pg/ml, P = 0.04). Also, following breakfast consumption AG levels stayed flat in elderly participants while they significantly decreased and then rose to preprandial levels in younger participants (time × age interaction effect P = 0.03) [91]. In a cross-sectional study comparing younger and older (mean age 24.2 ± 2.6 vs. 39.4 ± 6.9 y) lean (mean BMI 21.4 ± 0.31 vs. 21.9 ± 0.48 kg/m^2), overweight

(mean BMI 27.1 ± 0.47 vs. 27.4 ± 0.30 kg/m^2), and obese (mean BMI 34.7 ± 1.30 vs. 37.1 ± 1.0 kg/m^2) women, Schutte et al. showed that decreased fasting plasma ghrelin concentrations significantly correlated with increased age (r = –0.20, P < 0.05) [92]. Makovey et al. also showed a correlation, but not a strong one [93]. In a cross-sectional study that examined total ghrelin concentrations in younger and older participants (mean age 37.48 ± 8.97 vs. 59.15 ± 5.85 y, mean BMI 26.4 ± 4.9 vs. 27.3 ± 3.7 kg/m^2), it was reported that increased age significantly predicted decreased ghrelin levels after adjusting for gender, fat mass, and body size, but it only accounted for 15-20% of the variance (P < 0.05) [93]. However, in a randomized crossover trial on adults ranging in ages from 26 to 74 years (mean age 55.3 ± 2.9 y, mean BMI 29.2 ± 2.2 kg/m^2) that examined total plasma ghrelin concentrations following breakfast consumption versus a non-breakfast condition, Greenman et al. found no correlation between ghrelin levels and age [94]. Makovey et al., however, reported that lack of a significant correlation in the Greenman study was likely due to lack of power [93]. Greenman et al. included 24 participants in their study while Makovey et al. included 158 participants. Due to the fact that three studies did find a significant association between age and plasma ghrelin concentrations, particularly one in which AG concentrations were compared [91], recruitment for the proposed study will be limited to adult participants between the ages of 18 and 40 years. The upper limit of 40 years was chosen because it is the approximate median of 30 years and 51.2 years, the upper age limits used for the younger groups in the studies by Schutte et al. and Makovey et al., respectively.

2.6.2 Activity Level

Research has reported an effect of exercise on plasma ghrelin concentrations. It will, therefore, be important to consider activity level as a factor that potentially could impact appetite regulating

hormones. As evidence, two studies involving non-trained adults that compared changes in ghrelin concentrations between exercise and non-exercise conditions reported a positive association [95, 96]. Cheng et al. examined the acute effects of 50 minutes of moderate intensity exercise on appetite regulation in a randomized crossover trial [96]. In the study, young men (mean age 24.6 ± 4.8 y, mean BMI 25.4 kg/m^2) completed three trials: breakfast consumption followed by exercise, exercise followed by breakfast consumption, and breakfast consumption only. It was reported that pre-breakfast fasting plasma ghrelin concentrations significantly increased and pre-breakfast subjective hunger ratings significantly decreased one hour after exercise in the exercise followed by breakfast condition ($P \leq 0.05$ for both). Also, plasma ghrelin concentrations were significantly greater and subjective ratings of hunger were significantly lower after the breakfast consumption followed by exercise condition when compared with the breakfast consumption only condition ($P \leq 0.05$ for both) [96]. This study, though, investigated the acute effects of exercise on total plasma ghrelin. Only two studies have specifically investigated the acute effects of exercise on AG. In a randomized crossover trial on young men (mean age 21.2 ± 0.7 y, mean BMI 22.2 ± 0.7 kg/m^2), Broom et al. reported that the total AUC for AG concentrations was significantly greater following exercise than following the sedentary control condition (mean 510 vs. 317 pg/ml/3 h, $P = 0.021$; and mean 1401 vs. 917 pg/ml/9 h, $P = 0.033$) and that hunger ratings during the first three hours of the trials were significantly higher during the control condition than the exercise condition (mean score 32 vs. 24, $P = 0.013$) [95]. However, in another randomized crossover trial, Unick et al. reported that AG was unaltered by exercise (time × condition interaction effect $P = 0.16$), as were subjective hunger ratings (AUC for hunger scores, exercise: 395.5 ± 164.5 vs. resting: 391.7 ± 192.6, $P = 0.94$) [97]. None of the above studies, though, examined the long-term effects of exercise on

fasting plasma ghrelin concentrations. Therefore, it is difficult to conclude that the AG concentrations of sedentary individuals would respond to breakfast consumption differently than AG concentrations of individuals who regularly exercise.

Although there have been several research studies demonstrating the effects of exercise on GLP-1 concentrations in trained athletes and children [98-100], only the study by Unick et al. has examined the effects on non-trained adults [97]. The investigators reported that although the overall condition × time interaction effect was not significant ($P = 0.41$), the AUC for GLP-1 over the entire testing day was significantly lower after exercise (exercise: 402.4 ± 98.8 vs. rest: 422.8 ± 103.2 ng/mL × 120 min., $P < 0.05$). As with AG, though, no studies have examined the long-term effects of exercise on fasting GLP-1 concentrations, thus making it difficult to conclude that GLP-1 concentrations would respond to breakfast consumption differently between sedentary and active individuals. Regardless, research has demonstrated a significant association between physical activity and AG, GLP-1 and subjective hunger ratings. Thus, the level of physical activity of participants will need to be controlled in the proposed study so that an association between breakfast consumption and plasma AG and GLP-1 concentrations can be appropriately examined. To control for the effect of physical activity on plasma AG, plasma GLP-1, and subjective hunger ratings, recruitment will be limited to sedentary participants who engage in less than 30 minutes of moderate-intensity exercise per week. Physical activity will be estimated for both testing days. It will be self-reported by participants in a food and physical activity diary.

2.6.3 BMI

Research shows that fasting levels of plasma ghrelin and GLP-1 are different between normal weight, overweight, and obese individuals [27, 49, 84, 85, 94, 101, 102]. Plasma ghrelin levels are negatively correlated with BMI [49, 94, 102]. In a cross-sectional study and a randomized crossover trial on lean (mean BMI 25.4 ± 2.3 kg/m^2), overweight (mean BMI 29.2 ± 2.2 kg/m^2), and obese (mean BMI 38.2 ± 4.8 kg/m^2) adults (mean age 42.0 ± 5.6 y), Tschop et al. and Greenman et al., respectively, reported similar inverse correlations between increased BMI and decreased fasting total plasma ghrelin levels (r = –0.50, $P < 0.01$ and r = –0.47, $P = 0.02$, respectively) [49, 94]. In obese individuals, fasting levels of plasma ghrelin are lower and postprandial decreases are often attenuated when compared with normal weight individuals [49, 101-104]. In cross-sectional studies on normal weight (mean BMI 23.2 ± 0.8 kg/m^2), overweight (mean BMI 29.2 ± 2.2 kg/m^2), and obese (mean BMI 33.7 ± 2.7 kg/m^2) adults, both Tschop et al. and Shiiya et al. reported fasting plasma ghrelin concentrations in obese adults to be 32% lower than in normal weight adults ($P < 0.01$ and < 0.05, respectively) [49, 101]. Additionally, in an observational trial that examined differences in plasma ghrelin concentrations following breakfast in normal weight (mean BMI 22 ± 2.2 kg/m^2) and obese (mean BMI 33.8 ± 5.7 kg/m^2) adults, Erdmann et al. reported that the postprandial decrease in plasma ghrelin concentrations in obese participants was attenuated when compared with normal weight participants (–137.6 ± 107 pg/ml vs. –214.8 ± 247 pg/ml, respectively, $P < 0.001$) [103]. Plasma ghrelin levels also change with weight loss. In a weight loss intervention trial on adults, Cummings et al. demonstrated a 24% increase in the AUC for the 24-hour ghrelin profile following diet-induced weight loss of 17% of initial body weight over six months (2772 ± 334 pmol-days/L pre-weight loss vs. 3429 ±

429 pmol-days/L post-weight loss, $P = 0.006$) [27]. Shiiya et al. have proposed that ghrelin concentrations are inversely correlated with BMI and change with weight loss as part of a negative feedback mechanism to maintain energy homeostasis [101]. This would theoretically occur through up-regulation of ghrelin expression under conditions of negative energy balance, such as in times of starvation or high energy expenditure, and down-regulation under conditions of positive energy balance, such as obesity [101]. The reviewed studies have examined the effects of body weight on total plasma ghrelin. Only one study has compared fasting and postprandial levels of plasma AG between weight classes and found no correlation [97]. However, a non-breakfast condition was not a part of the research design. Because of that and due to the fact that the ratio of AG to DG can change while total plasma ghrelin concentrations stay the same, it is difficult to conclude that plasma AG levels correlate with BMI.

Like plasma ghrelin concentrations, research shows that GLP-1 concentrations are lower in obese individuals than normal weight individuals. For example, following a weight loss intervention trial on adult men, Verdich et al. reported that the total AUC for GLP-1 was lower in obese participants before and after weight loss compared to normal weight participants ($P < 0.05$), but following weight loss the response improved in reduced obese participants to 80-88% of that of normal weight participants ($P = 0.003$). The AUC for GLP-1 increased in a stepwise manner when comparing obese to reduced obese to normal weight participants ($P = 0.003$) [84]. Examining differences between weight classes in the responses of AG and GLP-1 to breakfast consumption is a primary aim of this study. An equal number of normal weight, overweight, and obese participants will be recruited into the study. Also, in an effort to control for the effect of changes in body weight on plasma AG and GLP-1 levels, recruitment will be limited to weight-stable women who have lost no more than 10 pounds over the six months prior to testing. The

10 pound limit was chosen as it was less than the lower end of the weight reduction range (5.3-32.7 kg) in the study by Verdich, et al. in which a significant change in the response of GLP-1 was noted after a six month weight loss intervention [84].

2.6.4 Frequency of Breakfast Consumption

Farshchi et al. reported that irregular meal frequency, including variability in breakfast consumption, disturbs energy metabolism [21, 105, 106]. This is mainly due to changes in the insulin response. In a randomized crossover study involving normal weight women (mean age 23.7 ± 7.4 y, mean BMI 22.4 ± 2.4 kg/m^2), the investigators reported a greater insulin response occurring after irregular meal patterns (a predetermined meal frequency varying between 3-9 meals/day for 14 days) than after regular meal patterns (6 occasions/day for meals and snacks) (P = 0.001) [105, 106]. Specifically to breakfast, they reported that the AUC of insulin profile showed a significantly greater response following a breakfast skipping period than after a breakfast consumption period [21]. This is important because since GLP-1 is known to have a strong incretin effect on glucose-dependent insulin secretion [22, 34], it is possible that an irregular breakfast consumption pattern could impact GLP-1 if it is also having an effect on the insulin response. Unfortunately, plasma GLP-1 concentrations were not an observed outcome in the Farshchi study. Based on the research of Farshchi et al., breakfast consumption frequency is a factor that needs to be controlled in future research. Farshchi et al. did not define breakfast consumption, but simply stated that only participants who usually ate breakfast were recruited for the study. However, in a cross-sectional study that examined the weight maintenance behaviors of NWCR participants who consume breakfast regularly (n = 2645) compared to individuals who do not consume breakfast regularly (n = 314), Wyatt et al. identified regular

breakfast consumers as those eating breakfast four or more times per week and non-regular breakfast consumers as those eating breakfast three or fewer days per week [16]. They reported no difference between groups in weight lost (32 vs. 34 kg, respectively, $P = 0.14$), duration of weight-loss maintenance (7.9 vs. 7.7 y, respectively, $P = 0.29$), or daily energy intake (1394 vs. 1366 kcals/day, respectively, $P = 0.50$). However, regular breakfast consumers did engage in slightly more physical activity than non-regular consumers (2657 vs. 2391 kcal/wk, respectively, $P = 0.05$). Based upon these results, for the proposed study recruitment will be limited to participants who consume breakfast at least 4 days per week.

2.6.5 Blood Sampling Timing

It is important that blood is sampled at appropriate times to monitor the changes in AG and GLP-1 concentrations. Based upon the previously reviewed research [26, 37, 38, 42, 57, 84, 85], AG is reported to reach nadir levels and GLP-1 is reported to reach peak levels within one hour of meal consumption. Both have been observed to then trend to preprandial levels within an additional hour. Also, it appears that even though circulating concentrations of AG and GLP-1 correlate with BMI, the temporal pattern of each is not impacted by body weight. As an example, Cummings et al. reported that although plasma ghrelin levels were greater in the normal weight controls than the obese participants, both before and after weight loss, the temporal pattern was similar at all time points across all weight class conditions when monitored over a 24-hour period [27].

In the reviewed literature that examined postprandial changes in ghrelin and GLP-1, plasma concentrations were commonly sampled for either two [85, 101, 107] or three hours [26, 48, 51, 84, 94]. The timing of blood samples varied between studies, occurring as frequently as

once every 15 minutes [84] to once per hour [48, 107]. Samples were commonly drawn at baseline, 30, 60, 90, and 120 minutes [51, 85, 94]. Based upon the research and the resources available to conduct the proposed study, blood will be sampled over two hours, once immediately before breakfast consumption and a non-breakfast waiting period and then at 30, 60, and 120 minutes after each testing condition.

2.7 CONCLUSIONS

It has been observed that individuals who have successfully maintained their body weight regularly consume breakfast [7]. It has been hypothesized that the hormonal response to breakfast consumption is a plausible mechanism to explain the impact of breakfast consumption on body weight [22-52], but this relationship has not been thoroughly examined. The orexigenic gut hormone acylated ghrelin and the anorexigenic gut hormone GLP-1 have been identified as two appetite-regulating hormones that are highly responsive to nutrient intake and play a role in glucose homeostasis [23, 24, 27, 28, 34, 41, 54, 68, 72-74, 79, 80]. They, therefore, may play a significant role in the pathway between breakfast consumption and successful maintenance of body weight. Results of studies examining the influence of breakfast consumption on acylated ghrelin and GLP-1 have been mixed, mostly due to differences in research methodology. This study will focus on the acute effect of breakfast consumption on acylated ghrelin and GLP-1. It is hypothesized that plasma AG concentrations will be significantly lower and plasma GLP-1 concentrations will be significantly higher following breakfast consumption than following a non-breakfast condition. This study will also explore and compare subjective ratings of hunger and satiety following both testing conditions and correlate them with plasma acylated ghrelin and

GLP-1 concentrations, respectively. Also, differences in self-reported levels of total daily energy intake between testing conditions will be examined as will the impact of BMI on plasma acylated ghrelin and GLP-1 concentrations following both testing conditions. Understanding the relationship between breakfast consumption and body weight will provide evidence to support a simple strategy to prevent and treat obesity

3.0 RESEARCH DESIGN AND METHODS

3.1 SUBJECTS

A total of 20 pre-menopausal women between the ages of 18 and 40 years who regularly eat breakfast were recruited to participate in this study. Only women were recruited because a gender effect is not a hypothesis of the study and the study was not powered to examine a gender effect. Women were chosen instead of men since 77% of participants in the NWCR are women [7]. Sedentary, younger women were used to control for the effects of exercise [39, 96, 108] and aging [91, 109] on AG and GLP-1. The sedentary condition was defined as less than 30 minutes of moderate-intensity aerobic exercise per week over the six months prior to recruitment. Only women who reported regular breakfast consumption were used to control for the effect of irregular meal frequency on energy metabolism and appetite-regulating hormones [21, 105, 106]. Regular breakfast consumption was defined as consuming breakfast four or more days per week based upon the data reported by Wyatt et al. in their cross-sectional study that described breakfast consumption practices of NWCR participants [16]. The independent variable breakfast was defined as consuming a food or mixture of foods that equals or exceeds the kilocalorie content of one serving of milk between the hours of 5:00 a.m. and 10:00 a.m. on weekdays and between 5:00 a.m. and 11:00 a.m. on weekends [11-13, 88]. Seven normal weight (BMI 18.5-24.9 kg/m^2), seven overweight (BMI 25.0-29.9 kg/m^2), and six Class I or Class II obese (BMI

30.0-39.9 kg/m^2) women were recruited so that the exploratory aim investigating the effect of BMI on the acute response of plasma AG and GLP-1 following a breakfast and non-breakfast condition could be examined, although the study was not powered to identify statistically significant differences.

Exclusionary criteria were:

1. Breakfast consumption < 4 days per week. Farshchi et al. have reported disturbances in energy metabolism following irregular breakfast consumption patterns, but did not define regular breakfast consumption [21, 105, 106]. Other research studies comparing breakfast consumption and non-breakfast conditions have considered participants who eat breakfast at least four times per week as regular breakfast consumers [14, 16, 63, 110].
2. Working between the hours of midnight and 8:00 a.m. Waking diurnal patterns may impact the response of AG and GLP-1 to meal consumption if monitored at a time when participants are normally eating supper than when breakfast is normally consumed [101, 111, 112]. Other research has also demonstrated an effect of sleep deprivation and daylight influence on plasma ghrelin levels [113, 114].
3. History of type 1 or type 2 diabetes. Both ghrelin and GLP-1 interact with insulin and glucose as part of a feedback loop. Irregular glycemic and insulin responses associated with diabetes can impact ghrelin and GLP-1 responses [28, 44, 56, 101, 102, 115].
4. Current diagnosis of an eating disorder. Research has reported an association between diagnosed bulimia nervosa [116] and anorexia nervosa [117, 118] and

significantly increased basal GLP-1 and basal ghrelin levels, respectively, when compared to healthy controls.

5. Currently pregnant or up to nine weeks post-partum and/or lactating. Research has reported that AG is significantly decreased during pregnancy and the percentage of total ghrelin that is AG is significantly greater up to nine weeks post-partum when compared to non-pregnant women [119, 120].
6. Currently on a weight-loss or weight-gain regimen. Research has reported that the ghrelin and GLP-1 responses are inversely associated with significant changes in weight. Both responses are down-regulated in obese individuals and increase with significant weight loss [27, 49, 84, 101, 103].
7. History of bariatric surgery. Research has reported that bariatric surgeries, such as Roux-en-Y gastric bypass and intragastric balloon, are associated with significantly decreased ghrelin responses and significantly increased GLP-1 responses [27, 121, 122].
8. Current use of medications that could affect weight or eating patterns (e.g. steroids). As above, the ghrelin and GLP-1 responses are inversely associated with significant changes in weight [49, 84].
9. Current diagnosis of a medical condition that could alter metabolism and weight (e.g. thyroid disease). As above, the ghrelin and GLP-1 responses are inversely associated with significant changes in weight [49, 84].

3.2 RECRUITMENT

Participants were recruited through internet sources and local flyers. After giving verbal consent, they underwent an initial telephone screening by a staff member of the University of Pittsburgh's Physical Activity and Weight Management Research Center (PAWMRC) to ensure eligibility requirements were met. During the telephone screening, BMI was calculated based on the participant's self-reported height and weight. Once seven participants were recruited in a weight class, additional potential participants within that weight class' BMI range were excluded. The BMI of each participant was confirmed by measurement of weight and height at the PAWMRC prior to testing.

Each participant attended an orientation session at which details of the study were reviewed and an opportunity to have questions answered was provided. At the session, participants completed a medical history questionnaire and a Stunkard and Messick Three-Factor Eating Questionnaire [123]. Height and weight were assessed so assignment to the recruited weight class could be verified and the kilocalorie amount of the breakfast meal could be planned. In addition, waist and hip measurements were taken and body fat was assessed via bioelectrical impedance analysis (BIA) for potential future analysis of differences in outcomes between weight classes. Prior to the initiation of in-person screening or experimental procedures, participants signed an informed consent document to verify their acknowledgement of the study procedures and their understanding of any associated risks with the study procedures. All study procedures were approved by the University of Pittsburgh Institutional Review Board.

3.3 STUDY DESIGN

Participants reported to the PAWMRC on two separate days within a two-week period, separated by at least 3 days. The study used a randomized crossover design in which participants were randomly assigned to one of two initial study conditions: breakfast consumption following an overnight fast or no breakfast following an overnight fast. The second testing session took place 3 to 14 days after the first session. Testing procedures were the same following each study condition. Both sessions were conducted between days 7 and 21 of the participant's menstrual cycle to control for the effect of hormones on outcome measures. At least 48 hours prior to the first testing session, participants received instructions in writing to refrain from vigorous exercise the day before and the morning of each testing session, and to fast for at least 12 hours the night before each testing session. Participants were also instructed to maintain regular eating patterns for at least two days prior to each testing visit. A phone call reminding participants of these requirements was made by a staff member from the PAWMRC at least 48 hours before each testing session.

3.3.1 Non-Breakfast Condition

On the day of testing participants reported to the PAWMRC between the hours of 7:00 a.m. and 9:00 a.m. having fasted since midnight. A staff member reviewed the testing procedures and the participant provided a urine sample to rule out pregnancy via a urine pregnancy test. Once it was confirmed that the participant was not pregnant, the participant underwent the initial blood draw. Immediately following the blood draw, the participant completed a hunger and satiety visual analog scale (VAS) questionnaire [124]. This time point was designated as "baseline." The

participant then was directed to the waiting area and sat for fifteen minutes, an amount of time similar to the time that was required to consume the breakfast meal during the breakfast consumption visit. However, the participant continued to fast. The participant then rested in a seated, upright position for two hours, having access to newspapers, magazines, and standardized videos that were provided by the investigators. The participant underwent additional blood draws and then completed additional hunger and satiety questionnaires at 30, 60, and 120 minutes after the 15-minute waiting period. These time points were designated as 30 minutes (30 min.), 60 minutes (60 min.), and 120 minutes (120 min.), respectively. Two hours after the 15-minute waiting period and immediately following the last blood draw (120 min.), the participant was provided with a standardized snack prior to leaving the PAWMRC. The intention of the snack was to provide a minimal amount of energy to the participant, who had fasted since at least midnight. In an effort to control for the impact of the snack on total daily energy intake, it was standardized to provide approximately 180-190 calories for all participants. Each participant was required to consume one Luna Bar or one can of Slim Fast Shake and was provided with bottled water. The participant had a variety of options to choose from, such as Lemon Zest, Nutz Over Chocolate, Peanut Butter Cookie, and S'mores Luna Bars or Milk Chocolate Slim Fast Shakes. All of the Luna Bars had a similar nutrient profile and provided 180 calories with 24-26 grams of carbohydrate (51-55% of calories), 8-9 grams of protein (18-20% of calories), and 5-6 grams of fat (25-30% of calories). The Slim Fast Shakes had a nutrient profile similar to the Luna Bars and provided 190 calories with 23-25 grams of carbohydrate (51-53% of calories), 10 grams of protein (21-22% of calories), and 6 grams of fat (28-30% of calories). When the participant was finished eating, a staff member of the PAWMRC assisted the participant in recording the food eaten during the testing session in a food and physical

activity diary. The staff member then reminded the participant to record all food intake and physical activity for the rest of the day in the food and physical activity diary and answered any questions the participant had (Figure 3).

3.3.2 Breakfast Consumption Condition

During the breakfast consumption visit the participant followed the same procedures as in the non-breakfast visit, but instead ate a breakfast meal in the first 15 minutes of the testing period. Once the procedures were reviewed and the urine sample was collected for the urine pregnancy test, the participant underwent the initial blood draw and completed a hunger and satiety questionnaire (baseline). The participant then was directed to the dining area in which a meal of a predetermined kilocalorie amount was provided. The breakfast meal provided 20% of the participant's estimated daily caloric needs based on resting energy expenditure (REE) multiplied by an activity factor of 1.3 for sedentary women [61, 125]. REE was determined using the Mifflin-St. Jeor formula with the participant's actual body weight [126]. The meal consisted of approximately 45-55% carbohydrate, 30-35% fat, and 15-20% protein [2]. The participant was required to completely eat the breakfast meal within 15 minutes.

Upon completion of the breakfast consumption period, the participant rested in a seated, upright position for the remainder of the two hours, having access to the same newspapers, magazines, and standardized videos that were provided by the investigators during the non-breakfast condition. Additional blood draws occurred and additional hunger and satiety questionnaires were completed by the participant at 30, 60, and 120 minutes after the breakfast consumption period [26, 45, 48, 51, 66, 107, 109]. Two hours after the breakfast consumption period and immediately following the last blood draw (120 min.), the participant was provided

with the same standardized snack as in the non-breakfast condition prior to leaving the PAWMRC. The snack was provided following the breakfast consumption condition as well in order to control for the impact of the snack on total daily energy intake following both testing conditions. Prior to leaving the PAWMRC, a staff member assisted the participant in recording the food eaten during the testing session in the food and physical activity diary. The staff member then reminded the participant to record all food intake and physical activity for the rest of the day in the food and physical activity diary and answered any questions the participant had (Figure 3).

3.4 COMPENSATION

Participants were compensated $300 for completing the study. To be eligible for the $300, the participant underwent four blood draws and completed four hunger and satiety questionnaires on each testing day. In addition, the participant recorded and was available to read back all food intake and physical activity entered in the two food and physical activity diaries. A staff member from the PAWMRC called the participant the day after each testing day and had the participant read back each entry from the diary. The staff member clarified details on any vague entries so that specific foods and activities, and amounts of each, were recorded accurately. Upon receipt of information from the second diary, the staff member thanked the participant for participating in the study and $300 was placed in an electronic payment account for the participant.

Step 1: RECRUITMENT

- Internet, local flyers

Step 2: SCREENING OF ELIGIBLE PARTICIPANTS

- Verbal consent obtained
- BMI calculated/assignment to weight class

Step 3: ORIENTATION MEETING

Review of study/ Questions answered → Completion of informed consent form → Medical history/ 3-Factor Eating Questionnaire → Height/Weight, Waist/Hip, BIA → Schedule visits 1 & 2

Step 4: SEND OUT INSTRUCTIONS (≥48 hours before Visit 1)

- Regular eating patterns/refrain from exercise 2 days prior to visit
- Fast after midnight the night before each visit

Step 5: REMINDER PHONE CALL (≥48 hours before each visit)

- Confirmed date of visit
- Reviewed instructions

Step 6: RANDOMIZATION & TESTING

- Randomized to Breakfast or Non-breakfast condition
- Testing conditions 3-14 days apart

Non-Breakfast

12:00am	7:00-9:00am	Baseline	30 min.	60 min.	120 min.	Post-testing
>Maintain regular eating pattern >Start fasting	>Report to PAWMRC >Review protocol	>Blood draw >Complete questionnaire >Waiting period >Read/watch videos	>Blood draw >Complete questionnaire >Read/watch videos	>Blood draw >Complete questionnaire >Read/watch videos	>Blood draw >Complete questionnaire	>Eat snack >Record in food & PA diary >Review food & PA diary

Breakfast

12:00am	7:00-9:00am	Baseline	30 min.	60 min.	120 min.	Post-testing
>Maintain regular eating pattern >Start fasting	>Report to PAWMRC >Review protocol	>Blood draw >Complete questionnaire >Breakfast >Read/watch videos	>Blood draw >Complete questionnaire >Read/watch videos	>Blood draw >Complete questionnaire >Read/watch videos	>Blood draw >Complete questionnaire	>Eat snack >Record in food & PA diary >Review food & PA diary

Figure 3. Study design

3.5 ASSESSMENT COMPONENTS

3.5.1 Height

Height was measured at the end of the orientation session after the participant signed an informed consent document. It was measured using a wall-mounted stadiometer and rounded to the nearest 0.1 cm. Height was used to calculate BMI and REE.

3.5.2 Body Weight

Body weight was measured at the end of the orientation session after the participant signed an informed consent document. It was measured using a digital scale and rounded to the nearest tenth of a pound. The participant was weighed in a hospital gown after removing all clothing except undergarments. Body weight was used to calculate BMI and REE.

3.5.3 Estimated Energy Expenditure

As has been done in past research that investigated the impact of breakfast on appetite-regulating hormones or body weight [25, 51, 61], the energy content of the breakfast test meal in the current study provided a percentage of the participant's estimated daily energy requirement. This is commonly done by using a published predictive formula to calculate estimated basal or resting energy expenditure and then multiplying the value by an activity factor. The breakfast meal in this study provided 20% of the participant's estimated daily caloric needs, calculated as REE multiplied by an activity factor of 1.3 for sedentary women [127]. Since a sedentary lifestyle

was part of the inclusion criteria for this study, an activity factor of 1.3 was used for all participants. REE was determined using the Mifflin-St. Jeor formula with the participant's actual body weight [126]. The Mifflin-St. Jeor formula for women is [126, 128]:

$$(9.99 \times \text{actual weight (kg)}) + (6.25 \times \text{height (cm)}) - (4.92 \times \text{age (y)}) - 161$$

While there are limitations in using predictive equations, research has reported that the Mifflin-St. Jeor formula most accurately measures REE [126, 128, 129]. Although noted limitations include underestimations in obese subjects and the elderly, estimations of REE using the Mifflin-St. Jeor formula matched estimation by indirect calorimetry more closely than other predictive equations for all population groups [128, 129].

In order to control for the impact of physical activity on energy expenditure, physical activity was estimated on both testing days. Physical activity was self-reported by participants in a food and physical activity diary. The time of day, type of activity, duration of activity, and intensity of activity was recorded in the diary. A staff member from the PAWMRC called the participant the day after each testing visit and had the participant read back each entry from the diary. The staff member clarified details on any vague entries so that specific activities and amounts were recorded accurately.

3.5.4 Test Meal

Participants consumed either a Luna Bar and whole milk or a toasted English muffin, cheddar cheese, and apple juice as the breakfast meal. In an effort to control for meal acceptance, participants were able to choose from one of several flavors of Luna Bars: Lemon Zest, Nutz Over Chocolate, Peanut Butter Cookie or S'mores. Each of these flavors has a similar macronutrient profile as previously outlined. One participant who was a vegan was provided

with hemp milk, which has a similar macronutrient profile to regular whole milk. One Luna Bar and four ounces of whole milk provided 255 calories and consisted of 30-32 grams of carbohydrate (47-50% of calories), 9-10 grams of fat (32-35% of calories), and 12-13 grams of protein (19-20% of calories) [130, 131]. For those who chose the toasted English muffin, cheddar cheese, and apple juice, one Thomas' English muffin, 1.25 ounces of cheddar cheese, and four ounces of apple juice provided 293 calories and consisted of 38.9 grams of carbohydrate (53.1% of calories), 10.4 grams of fat (31.9 % of calories), and 11.1 grams of protein (15.1% of calories) [131]. Amounts of the Luna Bar and milk or English muffin, cheddar cheese, and apple juice were increased or decreased proportionally by percent of total weight or volume to provide 20% of each participant's estimated daily caloric needs [12, 13, 51, 88]. The offerings for the breakfast meal were reviewed with participants during the initial telephone screening and at the orientation session.

3.5.5 Total Daily Energy Intake

Participants kept a detailed record of all foods consumed on each testing day. A standard food and physical activity diary was provided to each participant at each testing visit and a PAWMRC staff member instructed them on how to complete it. A specific description of each food item, measured or approximated amounts eaten, and the time at which each item was eaten was required. As detailed previously, on testing days a staff member of the PAWMRC assisted the participant in recording the food eaten during the testing session in the food and physical activity diary and reminded the participant to record all food intake and physical activity for the rest of the day in the diary. A staff member from the PAWMRC called the participant the day after each testing visit and had the participant read back each entry from the diary. The staff member

clarified details on any vague entries so that specific foods and activities and amounts of each were recorded accurately.

3.6 PRIMARY OUTCOME MEASURES

3.6.1 Blood Analysis

On each testing day, venous blood was collected and placed in collection tubes containing EDTA at each time point (baseline, 30, 60, and 120 min.) to analyze plasma levels of AG and GLP-1. For AG, 10 μl of p-hydroxymercuribenzoic acid (PHMB) was added per ml of blood to prevent the degradation of AG by protease. Samples were centrifuged at 1000G for 10 minutes at 4°C. The supernatant was transferred to a new tube and 100μl of 1N HCl was added per ml of plasma collected. The sample then was centrifuged at 3500 rpm for 5 minutes at 4°C and 1mL was aliquoted into a cryotube and stored at –70°C. The assay for AG was run using an ELISA kit from ALPCO (Salem, NH; cat # A05106).

The blood sample for GLP-1 was stored on ice and then centrifuged at 1000G for 10 minutes at 4°C. One milliliter of plasma then was aliquoted into a cryotube and stored at –70°C. The assay for GLP-1 was run using an ELISA kit from ALPCO (Salem, NH; cat # 43-GPTHU-E01). Analyses were performed at the Heinz Nutrition Laboratory in the Graduate School of Public Health at the University of Pittsburgh.

3.6.2 Total Daily Energy Intake

Self-reported total daily energy intake was compared between the breakfast consumption and non-breakfast conditions to investigate if breakfast consumption is associated with changes in total daily energy intake. It was hypothesized that self-reported levels of total daily energy intake would be significantly lower following breakfast consumption than following the non-breakfast condition. On each of the testing days, energy intake was determined by self-report using a 24-hour food and physical activity diary. To increase accuracy in documenting energy intake, prior to leaving the PAWMRC on each testing day a staff member assisted the participant in recording the food eaten during the testing session in the diary. Total energy intake for each day recorded was calculated using Diet Analysis Plus, version 10.0 nutrition analysis software (Cengage Learning, Independence, KY). All data was entered by the primary investigator. When an exact match was not available on Diet Analysis Plus for a prepared or convenience food item, restaurant or manufacturer websites were accessed to attain the information. If nutrition information was not available on the manufacturer's website, the nutrition information was first searched on CalorieKing.com and then on LiveStrong.com if necessary. If the information was still not available, the closest approximation to the actual item that was available on Diet Analysis Plus was used.

3.6.3 Hunger and Satiety

Hunger and satiety were rated on a 100 mm VAS questionnaire [124]. A copy is available in Appendix F. The left end of the scales for questions were anchored with phrases such as, "I am not hungry at all" or "I am completely empty," while the right end of the scales were anchored

with phrases such as, “I have never been more hungry” or “I am very satisfied.” Participants were instructed to make a mark across the scale line that best quantified their feelings of hunger or satiety. Quantification of hunger and satiety were determined by measuring the distance from the left end of the scale line to the mark made by the participant using a standard tape measure. Distances were rounded to the nearest millimeter.

3.7 STATISTICAL ANALYSIS

Descriptive analyses were performed for participants’ age, height, weight, BMI, waist girth, hip girth, waist/hip ratio, percent body fat, and human eating behavior construct scores as measured by the Three-Factor Eating Questionnaire. For each testing condition, mean and standard deviation were calculated for plasma AG and GLP-1 concentrations, calories from energy intake, and VAS scores for each of the hunger and satiety questions at each measured time point (baseline, 30, 60, and 120 min.) as well as for area under the curve (AUC).

A 4 × 2 two-way within-subjects repeated measures analysis of variance (ANOVA) (Time × Condition) was performed on plasma AG and GLP-1 concentrations as functions of the breakfast condition (breakfast consumption, non-breakfast) at each time point (baseline, 30, 60, 120 min.) to determine if significant differences exist. Main effects of Condition and Time and the interaction effect of Condition × Time are reported. Simple main effects were examined using a Bonferroni adjustment. The assumption of normality was tested using the Shapiro-Wilk test and the assumption of homogeneity of variance was tested using the Brown-Forsythe test.

Paired samples t-tests were used to examine differences between breakfast consumption and non-breakfast conditions for mean daily energy intake and for mean AUC of VAS scores for

each hunger and satiety question. A related-samples Wilcoxan signed rank test was used when data were not normally distributed. Also, to investigate if there was an association between VAS scores and plasma AG and GLP-1 concentrations and between VAS scores and daily energy intake, bivariate correlation analyses were performed for each testing condition using a Pearson correlation. Spearman rank correlation was performed when data were not normally distributed.

Although the study was not powered to examine the effect of breakfast consumption between BMI groups, a $4 \times 2 \times 3$ three-factor ANOVA was performed on plasma AG and GLP-1 concentrations as functions of breakfast condition (breakfast consumption, non-breakfast), time (baseline, 30, 60, 120 min.) and BMI classification (normal weight, overweight, obese) to examine whether the pattern of differences in hormone concentrations between testing conditions was significantly different between BMI groups. The within-subjects independent variables were breakfast condition and time, and the between-subjects independent variable was BMI class. Main effects of Condition, Time, and BMI and the interaction effects of Condition $\times$ Time, Condition $\times$ BMI, Time $\times$ BMI, and Condition $\times$ Time $\times$ BMI are reported. In addition, a 3×2 mixed ANOVA (BMI $\times$ Condition) was performed on energy intake as a function of breakfast condition and BMI classification to examine whether the pattern of differences in energy intake between testing conditions was significantly different between BMI groups. The within-subjects independent variable was breakfast condition and the between-subjects independent variable was BMI class. When a significant interaction effect involving BMI was identified in any of the above analyses, the analyses were performed again to compare BMI groups two at a time to examine differences between BMI groups. The assumption of normality for each ANOVA analysis was tested using the Shapiro-Wilk test and the assumption of homogeneity of variance was tested using the Brown-Forsythe test. All of the above analyses

were performed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL) with the alpha level set at $P < 0.05$.

3.8 POWER ANALYSIS

The primary aim of this study was to investigate if breakfast consumption had a significant impact on the acute response of plasma AG and plasma GLP-1 concentrations over a two hour period, as well as an impact on total daily energy intake. A power analysis was performed to estimate an appropriate sample size for the study based on within-subjects, repeated measures ANOVA and based on matched pairs, two dependent means t-tests. In order to detect a moderate to large effect size with statistical power set at 0.80 and alpha at 0.05, 19 subjects needed to be recruited. Due to the possibility that participants would have incomplete data, an additional two subjects were added to the sample size to ensure adequate statistical power. Therefore, attempts were made to recruit a total of 21 subjects for this study. However, only 20 participants were able to be recruited with the resources available for recruitment.

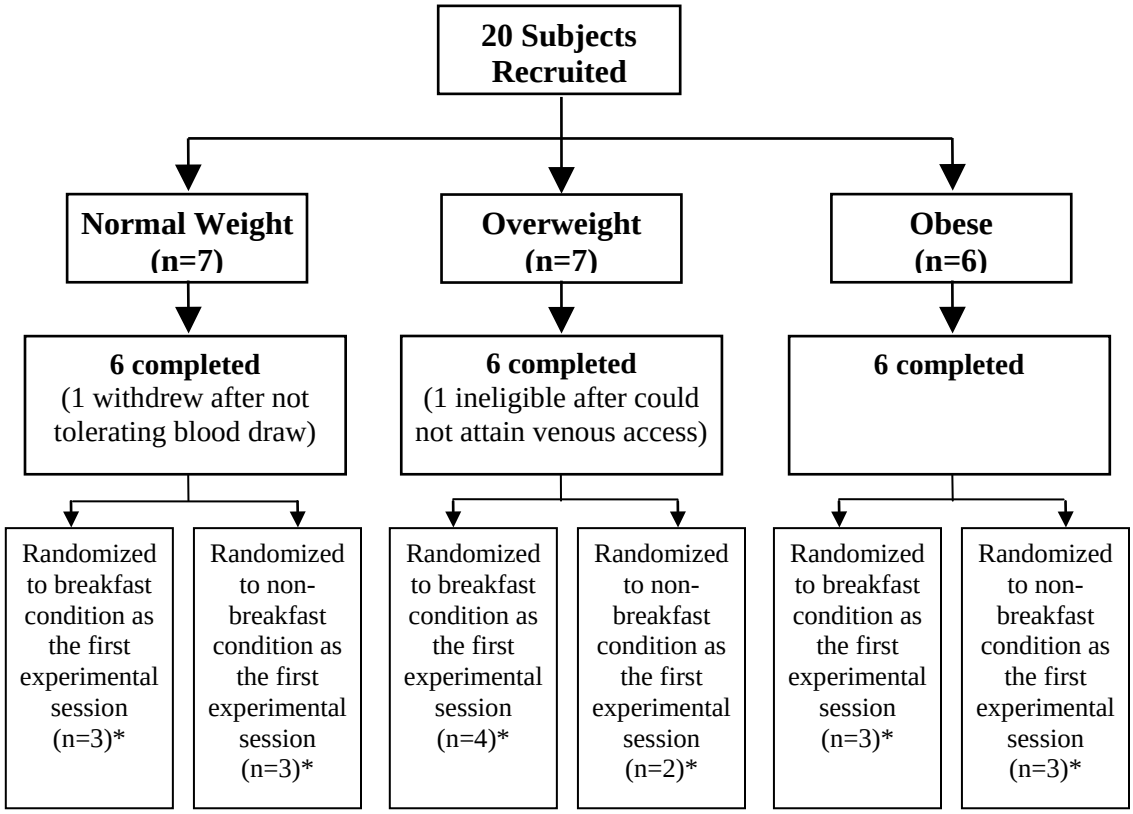
4.0 RESULTS

4.1 SUBJECTS

Twenty women (BMI: 26.8 ± 5.9 kg/m^2; age: 26.3 ± 6.0 years) consented to participate in the study. Seven participants were classified as normal weight (BMI: 18.5-24.9 kg/m^2), seven as overweight (BMI: 25.0-29.9 kg/m^2) and six as obese (30.0-39.9 kg/m^2). Participants were randomized to either the breakfast condition or non-breakfast condition as the first experimental session upon entry into the study, with BMI category not considered in the randomization scheme. Thus, 10 participants were randomized to perform the breakfast condition as the first experimental session and 8 participants were randomized to receive the non-breakfast condition as the first experimental session. Two participants withdrew from the study. One normal weight participant did not tolerate the blood draw at the first testing session and decided not to continue with the study. One overweight participant became ineligible after venous access could not be attained during the baseline blood drawing at the first testing session. Complete data was collected on a total of 18 participants (Figure 4).

Descriptive statistics (mean ± standard deviation) for the total sample and for each BMI group are shown in Table 1. A series of one-way analyses of variance (ANOVA) revealed that there were no significant differences between the BMI groups for age, height, and cognitive restraint, disinhibition and trait hunger constructs as measured by the Stunkard and Messick

Three-Factor Eating Questionnaire. By study design there were significant differences between BMI groups for body weight, BMI, waist girth, hip girth, waist-hip ratio, and body composition expressed as percent body fat ($P < 0.05$). However, differences between BMI groups for waist-hip ratio measured at the iliac crest level were not statistically significant ($P = 0.08$). A series of paired- samples t-tests revealed that there were no significant differences between height, weight, and body composition measurements for participants between testing visits (Table 2).



*Indicates that randomization occurred at the level of N=20 and BMI was not considered in the randomization scheme

Figure 4. Study enrollment and randomization

Table 1. Descriptive statistics by total sample and by BMI group (mean ± standard deviation)

	All groups (n=18)	**Normal Wt. (n=6)**	**Overweight (n=6)**	**Obese (n=6)**	***P*-value***
Age(y)	26.3 ± 6.0	25.7 ± 7.2	23.7 ± 2.1	29.7 ± 6.6	.220
Height (cm)	164.6 ± 4.4	162.8 ± 2.3	166.8 ± 5.2	164.6 ± 4.6	.283
Weight (lbs)	159.9 ± 37.7	117.3 ± 7.1	162.7 ± 10.0	198.9 ± 25.6	<.001[A,B,C]
BMI (kg/m^2)	26.8 ± 5.9	20.2 ± 1.2	26.6 ± 1.3	33.3 ± 3.0	<.001[A,B,C]
Waist Girth (cm):					
umbilicus[D]	94.5 ± 17.0	75.7 ± 5.7	96.4 ± 5.1	111.3 ± 9.6	<.001[A,B,C]
iliac crest[E]	91.3 ± 15.1	74.6 ± 5.6	91.8 ±5.3	106.1 ± 9.2	<.001[A,B,C]
Hip Girth (cm)	105.4 ± 13.7	91.8 ± 2.1	106.8 ± 3.5	118.4 ± 12.6	<.001[A,B]
Waist-Hip Ratio:					
umbilicus[D]	0.89 ± .07	.83 ± .07	.90 ± .07	.94 ± .04	.016[B]
iliac crest[E]	0.86 ± .06	.81 ± .07	.86 ± .07	.90 ± .04	.080
Body Fat (%)	46.7 ± 4.2	43.1 ± 2.2	46.7 ± 3.8	50.3 ±3.9	.008[B]
Human Eating Behavior Constructs (score):					
Cognitive Restraint	6.78 ± 4.3	5.83 ± 4.0	6.67 ± 3.6	7.83 ± 5.6	.742
Disinhibition	5.89 ± 3.3	4.33 ± 2.6	5.83 ± 4.0	7.50 ± 3.1	.274
Hunger	6.11 ± 4.0	5.67 ± 3.8	7.33 ± 4.1	5.33 ± 4.4	.675
Ethnicity (%):					
Hispanic/Latino	5.6	16.7	0.0	0.0	
Non-Hispanic/Latino	94.4	83.3	100.0	100.0	
Race (%):					
Asian	5.6	16.7	0.0	0.0	
Black/African American	16.6	0.0	16.7	33.3	
White	66.7	66.6	83.3	50.0	
Other/Mixed	11.1	16.7	0.0	16.7	

*One-way ANOVA computed with post-hoc analysis using a Bonferroni adjustment
[A] Normal Weight is significantly different than Overweight ($P < 0.05$)
[B] Normal Weight is significantly different than Obese ($P < 0.05$)
[C] Overweight is significantly different than Obese ($P < 0.05$)
[D] Waist girth measured at the level of the umbilicus
[E] Waist girth measured at the level of the iliac crest

Table 2. Descriptive statistics for breakfast and non-breakfast testing conditions (mean ± standard deviation)

n=18	Non-Breakfast Condition	Breakfast Condition	*P*-value
Height (cm)	164.6 ± 4.3	164.7 ± 4.3	.178
Weight (lbs)	160.1 ± 37.9	159.6 ± 37.6	.307
BMI (kg/m^2)	26.8 ± 6.0	26.7 ± 5.8	.270
Waist Girth (cm): umbilicus[A] iliac crest[B]	 93.8 ± 16.4 90.6 ± 15.3	 94.5 ± 16.4 90.8 ± 14.8	 .556 .744
Hip Girth (cm)	105.4 ± 13.4	105.6 ± 13.3	.472*
Waist-Hip Ratio: umbilicus[A] iliac crest[B]	 0.89 ± .06 0.86 ± .06	 0.89 ± .08 0.86 ± .07	 .685 .892
Body Fat (%)	46.8 ± 4.3	46.7 ± 4.4	.491

*Paired-samples Wilcoxan signed rank test used
[A] Waist girth measured at the level of the umbilicus
[B] Waist girth measured at the level of the iliac crest

4.2 ANALYSIS OF DATA BY SPECIFIC AIM

4.2.1 Specific Aim 1: Comparison of Changes in Plasma Acylated Ghrelin Concentrations Following Breakfast and Non-Breakfast Testing Conditions

Three participants had incomplete data for the acylated ghrelin (AG) measurements. One had a missing blood sample at the 60-minute time point of the Breakfast condition due to difficulty attaining venous access. The other two had compromised blood samples that could not be properly processed. Data screening of plasma AG concentrations identified that the assumption of normality was violated at the 120-minute time point of the Breakfast condition and at the 30-,

60-, and 120-minute time points of the Non-Breakfast condition. Data screening also identified one outlier whose samples deviated from the normal range of values at four different time points.

A 4 × 2 two-way within-subjects repeated measures ANOVA (Time × Condition) was performed on mean plasma AG concentrations. The three participants with missing data were not included in the analysis by the nature of the repeated measures test. The assumption of sphericity was not met for Time (Mauchly's $W = 0.049$, $P < 0.001$) or for Condition × Time (Mauchly's $W = 0.090$, $P < 0.001$). Thus, to examine the main effect of Time and the interaction effect, the Huynh-Feldt adjustment was used. There was a significant Condition × Time interaction effect ($P = 0.003$), indicating that the pattern of change in plasma AG concentrations across time differed between the experimental conditions. Data are presented in Table 3. Contributing to this significant interaction effect was the finding that the 30-minute and 60-minute concentrations of AG for the Breakfast condition were significantly lower than the baseline concentration ($P < 0.001$). Moreover, there was a significant difference in AG concentrations between the Non-Breakfast and Breakfast conditions at the 30-, 60-, and 120-minute time points ($P \leq 0.001$).

Additional analyses were performed to examine whether these findings were robust. Due to the non-normal distribution of some of the data, the analysis was repeated using transformed data (logarithmic transformation and square root transformation), and the pattern of the results was unchanged (data not presented). The analysis was performed again with the outlier participant removed from the sample, and the Condition × Time interaction effect and the main effect of Condition remained statistically significant (Table 3).

Table 3. Differences in plasma acylated ghrelin and GLP-1 concentrations between breakfast and non-breakfast testing conditions at each measured time point (mean ± standard deviation)

		Experimental Conditions		*P*-values		
Variable	**Testing Time**	**Non-Breakfast Condition**	**Breakfast Condition**	**Condition**	**Time**	**Condition × Time**
Acylated Ghrelin (pg/ml) (n=15)	Baseline	100.3 ± 51.8	110.0 ± 54.7	<0.001	0.100	0.003
	30 minutes	110.2 ± 65.4	64.9 ± 43.4[A,C]			
	60 minutes	117.1 ± 86.4	57.0 ± 44.6[A,C]			
	120 minutes	124.4 ± 91.5	90.2 ± 82.0[A]			
Acylated Ghrelin (pg/ml) (n=14)*	Baseline	97.4 ± 52.4	100.8 ± 43.3	<0.001	<0.001	<0.001
	30 minutes	97.1 ± 42.4	58.2 ± 36.2[A,D,E]			
	60 minutes	97.1 ± 39.9	49.9 ± 36.3[A,C,E]			
	120 minutes	103.0 ± 39.8	71.4 ± 38.8[A]			
GLP-1 (pm/L) (n=17)	Baseline	1.6 ± 1.7	1.8 ± 2.3	<0.001	0.331	0.042
	30 minutes	1.4 ± 1.6	2.3 ± 1.7[A]			
	60 minutes	1.4 ± 1.6	2.5 ± 1.9[A]			
	120 minutes	1.4 ± 1.5	2.2 ± 1.6[B]			
GLP-1 (pm/L) (n=16)*	Baseline	1.3 ± 0.9	1.3 ± 0.6	<0.001	0.031	0.002
	30 minutes	1.0 ± 0.7	2.0 ± 1.1[A]			
	60 minutes	1.1 ± 0.6	2.2 ± 1.5[A]			
	120 minutes	1.1 ± 0.7	1.9 ± 0.9[B]			

[A] Indicates that time point for Non-Breakfast condition is significantly different than the same time point for the Breakfast condition at $P \leq 0.001$

[B] Indicates that time point for Non-Breakfast condition is significantly different than the same time point for the Breakfast condition at $P < 0.05$

[C] Indicates significantly different than baseline at $P \leq 0.001$

[D] Indicates significantly different than baseline at $P < 0.05$

[E] Indicates significantly different than 120 minutes at $P < 0.05$

* Indicates that outlier was removed from the analysis

4.2.2 Specific Aim 2: Comparison of Changes in Plasma Glucagon-Like Peptide 1 Concentrations Following Breakfast and Non-Breakfast Testing Conditions

As mentioned above, one participant had a missing blood sample at the 60-minute time point of the Breakfast condition due to difficulty attaining venous access. Data screening of plasma glucagon-like peptide 1 (GLP-1) concentrations identified that the assumption of normality was violated at each time point of both testing conditions. Data screening also identified one outlier whose samples deviated from the normal range of values at each time point of both conditions.

A 4 × 2 two-way within-subjects repeated measures ANOVA (Time × Condition) was performed on mean plasma GLP-1 concentrations. The participant with missing data was not included in the analysis by the nature of the repeated measures test. The assumption of sphericity was not met for Time (Mauchly's $W = 0.389$, $P = 0.016$) or for Condition × Time (Mauchly's $W = 0.124$, $P < 0.001$). Thus, to examine the main effect of Time and the interaction effect, the Huynh-Feldt adjustment was used. There was a significant Condition × Time interaction effect ($P = 0.042$), indicating that the pattern of change in plasma GLP-1 concentrations across time differed between the experimental conditions. Data are presented in Table 3. Contributing to this significant interaction effect was the finding that there was a significant difference in GLP-1 concentrations between the Non-Breakfast and Breakfast conditions at the 30-, 60-, and 120-minute time points ($P \leq 0.05$).

Additional analyses were performed to examine whether these findings were robust. Due to the non-normal distribution of the data, the analysis was repeated using transformed data (logarithmic transformation), and the pattern of the results was unchanged (data not presented). The analysis was performed again with the outlier participant removed from the sample, and the

Condition × Time interaction effect and the main effect of Condition remained statistically significant (Table 3).

4.2.3 Specific Aim 3: Comparison of Changes in Daily Energy Intake Following Breakfast and Non-Breakfast Testing Conditions

Total daily intake was analyzed, which included the breakfast and/or snack provided to participants by the researchers on testing days plus all other discretionary intake recorded by participants for the rest of each testing day. Mean breakfast intake was 373 ± 44 kcals. Data screening identified that the assumption of normality was violated for the Non-Breakfast condition. It still was not met after performing logarithmic and square root transformations. Data screening also identified two outliers whose total daily intake deviated from the normal range of values for the Non-Breakfast condition.

A related-samples Wilcoxan signed rank test was performed to compare differences in total daily intake between testing conditions. The median of differences of total daily intake between conditions was not significant (P = 0.199) (Table 4). When the two outliers were removed from the sample, the assumption of normality was met for the Non-Breakfast condition. A paired samples t-test then was performed, and the difference still was not significant (P = 0.099) (Table 5).

Snack and discretionary intake was analyzed, which included the snack provided to participants by the researchers on testing days plus all other discretionary intake recorded by participants for the rest of each testing day, but did not include the breakfast meal provided to participants by the researchers. A related-samples Wilcoxan signed rank test was performed to compare differences in snack and discretionary intake between testing conditions. The median

of differences of snack and discretionary intake between conditions was not significant (P = 0.184) (Table 4). With the outliers removed from the sample, a paired samples t-test then was performed and the difference still was not significant (P = 0.484) (Table 5).

Snack intake alone was also analyzed. Although participants were required to consume a snack consisting of 180-190 kcals before leaving the research center on each testing day, two participants refused the snack and two participants did not completely eat the snack on the breakfast testing day, each citing that they were not hungry after consuming breakfast. On the non-breakfast testing day, one participant would not completely eat the snack. Data screening identified that the assumption of normality was violated for both Breakfast and Non-Breakfast conditions. The assumption of normality still was not met after transforming the data and after removing the previously mentioned outliers. Thus, only a related-samples Wilcoxan signed rank test was performed to compare differences in snack intake between testing conditions. The median of differences of snack intake between conditions was not significant (P = 0.089) (Table 4).

Lastly, discretionary intake was analyzed, which included only the intake recorded by participants after leaving the research center each testing day, and did not include intake from the breakfast meal and snack provided to the participants by the researchers. Data screening identified that the assumption of normality was violated for the Non-Breakfast condition. It still was not met after performing logarithmic and square root transformations. Therefore, a related-samples Wilcoxan signed rank test was performed to compare differences in discretionary intake between conditions. The median of differences of discretionary intake between conditions was not significant (P = 0.306) (Table 4). With the outliers removed from the sample, a paired samples t-test was performed and the difference still was not significant (P = 0.620) (Table 5).

Table 4. Comparison of energy intake for breakfast and non-breakfast testing conditions using the complete sample (median), n=18

Intake Level	Non-Breakfast Condition (kcals)	Breakfast Condition (kcals)	*P*-value*
Total Daily Intake[A]	1,596	1,883	.199
Snack and Discretionary Intake[B]	1,596	1,487	.184
Snack Intake[C]	180	180	.089
Discretionary Intake[D]	1,411	1,355	.306

*Related-samples Wilcoxan signed rank test computed

[A] Includes breakfast and/or snack provided by researchers and all other discretionary intake

[B] Includes only snack provided by researchers and all other discretionary intake

[C] Includes only snack provided by researchers

[D] Includes only discretionary intake recorded by participants, but not breakfast meal and snack provided by researchers

Table 5. Comparison of energy intake for breakfast and non-breakfast testing conditions using sample without outliers (mean ± standard deviation), n=16

Intake Level	Non-Breakfast Condition (kcals)	Breakfast Condition (kcals)	*P*-value*
Total Daily Intake[A]	1,560 ± 221	1,824 ± 555	.099
Snack and Discretionary Intake[B]	1,560 ± 221	1,453 ± 546	.484
Discretionary Intake[C]	1,384 ± 233	1,309 ± 552	.620

*Paired-samples t-test computed

[A] Includes breakfast and/or snack provided by researchers and all other discretionary intake

[B] Includes only snack provided by researchers and all other discretionary intake

[C] Includes only discretionary intake recorded by participants, but not breakfast meal and snack provided by researchers

4.2.4 Specific Aim 4: Comparison of the Influence of Body Mass Index on Changes in Plasma Acylated-Ghrelin and GLP-1 Concentrations Following Breakfast and Non-Breakfast Testing Conditions

Acylated Ghrelin

A 4 × 2 × 3 three-factor ANOVA (Time × Condition × BMI) was performed to examine whether the pattern of differences in AG concentrations between testing conditions was significantly different between BMI groups. The three participants with missing data were not included in the analysis by the nature of the repeated measures test. Data screening of plasma AG concentrations identified that the assumption of normality was violated at the 60-minute and 120-minute time points of the Non-Breakfast condition for normal weight participants. The assumption of sphericity was not met for Time (Mauchly's $W = 0.047$, $P < 0.001$) or for Condition × Time (Mauchly's $W = 0.089$, $P < 0.001$). Thus, to examine the main effect of Time and the interaction effect, the Huynh-Feldt adjustment was used. As mentioned above there was a significant Condition × Time interaction effect ($P = 0.001$), but no other interaction effects were significant. Data are presented in Table 6. The analysis was performed again with the previously mentioned outlier participant removed from the sample. The main effect of Time then was significant ($P < 0.001$) as was the main effect of BMI ($P = 0.034$), but all of the interaction effects remained not significant (data not presented).

Table 6. Comparison of changes in plasma acylated ghrelin concentrations by BMI group (mean ± standard deviation)

	Time Point	BMI Class	Non-Breakfast Condition	Breakfast Condition	Condition	Time	BMI	Condition × Time	Condition × BMI	Time × BMI	Condition × Time × BMI
Acylated Ghrelin (pg/ml)	Baseline	NW[A]	87.6 ± 39.5	122.1 ± 75.6	<0.001	0.085	0.273	0.001	0.654	0.306	0.233
		OW[B]	145.9 ± 55.2	130.4 ± 25.4							
		OB[C]	67.5 ± 25.3	77.4 ± 45.9							
		Total	100.3 ± 51.8	110.00 ± 54.7							
	30-minute	NW	129.3 ± 96.2	75.1 ± 52.9							
		OW	135.4 ± 30.2	79.1 ± 32.4							
		OB	65.9 ± 33.0	40.5 ± 40.3							
		Total	110.2 ± 65.4	64.9 ± 43.4							
	60-minute	NW	145.2 ± 142.7	60.5 ± 57.9							
		OW	132.4 ± 25.8	82.9 ± 33.1							
		OB	73.7 ± 38.7	27.7 ± 24.2							
		Total	117.1 ± 86.4	57.0 ± 44.6							
	120-minute	NW	155.9 ± 152.2	126.2 ± 131.6							
		OW	133.5 ± 25.4	98.9 ± 29.2							
		OB	83.8 ± 45.7	45.5 ± 34.2							
		Total	124.4 ± 91.5	90.2 ± 82.0							

[A] Normal Weight (n=5), [B] Overweight (n=5), [C] Obese (n=5)

Table 7. Comparison of changes in plasma GLP-1 concentrations by BMI group (mean ± standard deviation)

	Time Point	BMI Class	Non-Breakfast Condition	Breakfast Condition	Condition	Time	BMI	Condition × Time	Condition × BMI	Time × BMI	Condition × Time × BMI
GLP-1 (pm/L)	Baseline	NW[A]	2.5 ± 2.5	2.9 ± 3.7	<0.001	0.330	0.272	0.046	0.093	0.270	0.322
		OW[B]	1.2 ± 0.8	1.4 ± 0.5							
		OB[C]	1.0 ± 0.9	1.2 ± 0.6							
		Total	1.6 ± 1.7	1.8 ± 2.3							
	30-minute	NW	2.0 ± 2.6	3.2 ± 2.2							
		OW	1.1 ± 0.8	1.7 ± 0.9							
		OB	1.0 ± 0.8	1.9 ± 1.3							
		Total	1.4 ± 1.6	2.3 ± 1.7							
	60-minute	NW	2.0 ± 2.6	4.1 ± 2.4							
		OW	1.2 ± 0.6	1.7 ± 0.6							
		OB	1.0 ± 0.8	1.5 ± 1.0							
		Total	1.4 ± 1.6	2.5 ± 1.9							
	120-minute	NW	2.0 ± 2.4	3.0 ± 2.2							
		OW	1.2 ± 0.8	1.8 ± 0.7							
		OB	1.0 ± 0.7	1.7 ± 1.0							
		Total	1.4 ± 1.5	2.2 ± 1.6							

[A] Normal Weight (n=6), [B] Overweight (n=5), [C] Obese (n=6)

GLP-1

Similar analyses were performed on plasma GLP-1 concentrations. The participant with missing data was not included in the analysis by the nature of the repeated measures test. As previously mentioned, data screening of GLP-1 concentrations identified that the assumption of normality was violated at each time point of both testing conditions. The assumption of sphericity was not met for Time (Mauchly's $W = 0.233$, $P = 0.002$) or for Condition × Time (Mauchly's $W = 0.087$, $P < 0.001$). Thus, to examine the main effect of Time and the interaction effect, the Huynh-Feldt adjustment was used. As mentioned above there was a significant Condition × Time interaction effect ($P = 0.046$), but no other interaction effects were significant. Data are presented in Table 7.

The analysis was performed again with the previously mentioned outlier participants removed from the sample. The interaction effect of Condition × Time × BMI then was significant ($P < 0.001$), as was the main effect of Time ($P = 0.014$). To determine the extent of the interaction effect between BMI groups, a 4 × 2 × 2 three-factor ANOVA (Time × Condition × BMI) was performed between BMI groups two at a time. The interaction effect of Condition × Time × BMI was significant between normal weight and overweight participants ($P = 0.003$) and between normal weight and obese participants ($P = 0.001$), but not between overweight and obese participants ($P = 0.71$) (Data not presented).

Energy Intake

Although not a specific aim of the study, a 3 × 2 mixed ANOVA (BMI × Condition) was performed to examine whether the pattern of differences in energy intake between testing conditions was significantly different between BMI groups. A separate analysis was performed

on total daily intake, snack and discretionary intake, and discretionary intake only. Data screening identified that the assumption of normality was met for all intake categories for each testing condition at each BMI classification level. There was a significant Condition × BMI interaction effect for each intake level (total, $P = 0.019$; snack and discretionary, $P = 0.021$; and discretionary only, $P = 0.019$). To determine the extent of the interaction effect between BMI groups, a 2 × 2 mixed ANOVA (BMI × Condition) was performed between BMI groups two at a time. The interaction effect of Condition × BMI was significant between normal weight and overweight participants at each intake level (total, $P = 0.019$; snack and discretionary, $P = 0.026$; and discretionary only, $P = 0.025$) and between overweight and obese participants at each intake level (total, $P = 0.045$; snack and discretionary, $P = 0.038$; and discretionary only, $P = 0.036$), but not between normal weight and obese participants (total, $P = 0.174$; snack and discretionary, $P = 0.222$; and discretionary only, $P = 0.193$). Data are presented in Table 8. Contributing to this significant interaction effect was the finding that intake following the Breakfast condition was lower or similar to intake following the Non-Breakfast condition for normal weight and obese participants, while for overweight participants intake was higher following the Breakfast condition than following the Non-Breakfast condition. However, the main effect of Condition was only significant for total daily intake. The analyses were performed again with the outlier participants removed from the sample and the main effect of Condition then was not significant for total daily intake ($P = 0.087$), nor was the interaction effect of Condition × BMI between overweight and obese participants for total daily intake ($P = 0.060$) or discretionary intake only ($P = 0.052$) (Data not presented).

Table 8. Comparison of changes in energy intake between breakfast and non-breakfast testing conditions by BMI group (mean ± standard deviation)

							BMI Group-by-Group Interaction Effects		
Intake Level	**BMI Class**	**Non-Breakfast Condition (kcals)**	**Breakfast Condition (kcals)**	**Condition**	**BMI**	**Condition × BMI**	**NW[D] vs OW[E]**	**NW vs OB[F]**	**OW vs OB**
Total Daily Intake[A]	NW[D]	1,593 ± 226	1,588 ± 314	0.036	0.288	0.019	0.019	0.174	0.045
	OW[E]	1,470 ± 162	2,236 ± 649						
	OB[F]	2,049 ± 669	2,061 ± 856						
	Total	1,704 ± 469	1,962 ± 669						
Snack and Discretionary Intake Only[B]	NW	1,593 ± 226	1,267 ± 315	0.315	0.364	0.021	0.026	0.222	0.038
	OW	1,470 ± 162	1,852 ± 637						
	OB	2,049 ± 669	1,647 ± 872						
	Total	1,704 ± 469	1,589 ± 659						
Discretionary Intake Only[C]	NW	1,413 ± 230	1,100 ± 340	0.436	0.319	0.019	0.025	0.193	0.036
	OW	1,290 ± 162	1,702 ± 640						
	OB	1,881 ± 669	1,519 ± 841						
	Total	1,528 ± 473	1,440 ± 655						

[A] Includes breakfast and/or snack provided by researchers and all other discretionary intake
[B] Includes only snack provided by researchers and all other discretionary intake
[C] Includes only discretionary intake recorded by participants, but not breakfast meal and snack provided by researchers
[D] NW = Normal Weight (n=6)
[E] OW = Overweight (n=6)
[F] OB = Obese (n=6)

4.3 EXPLORATORY ANALYSES

4.3.1 Comparison of Subjective Ratings of Hunger and Satiety Following Breakfast and Non-Breakfast Testing Conditions

Mean area under the curve (AUC) for scores from each of the five hunger and satiety VAS questions were compared between testing days to examine differences in subjective ratings of hunger and satiety. Data screening identified that the assumption of normality was violated for question 3 of the Non-Breakfast condition and that there was an outlier whose ratings deviated from the normal range on questions 2 and 3 of the Non-Breakfast condition.

Paired-samples t-tests were performed on AUC scores for questions 1, 2, 4 and 5 and a related-samples Wilcoxan signed-rank test was performed on question 3. Questions 1 and 4 assessed participants' feelings of hunger, questions 2 and 3 assessed participants' feelings of satiety, and question 5 assessed participants' feelings of thirst. A copy of the questionnaire is available in Appendix F. Mean hunger ratings were significantly lower for the Breakfast condition than for the Non-Breakfast condition ($P < 0.001$, each). Mean satiety ratings were significantly higher for the Breakfast condition than for the Non-Breakfast condition ($P < 0.001$, each). Lastly, mean ratings of thirst were significantly lower for the Breakfast condition than for the Non-Breakfast condition ($P = 0.002$). Data are presented on Figure 5. The analyses were performed again with the outlier participant removed from the sample, and the significance of the results did not change (data not presented).

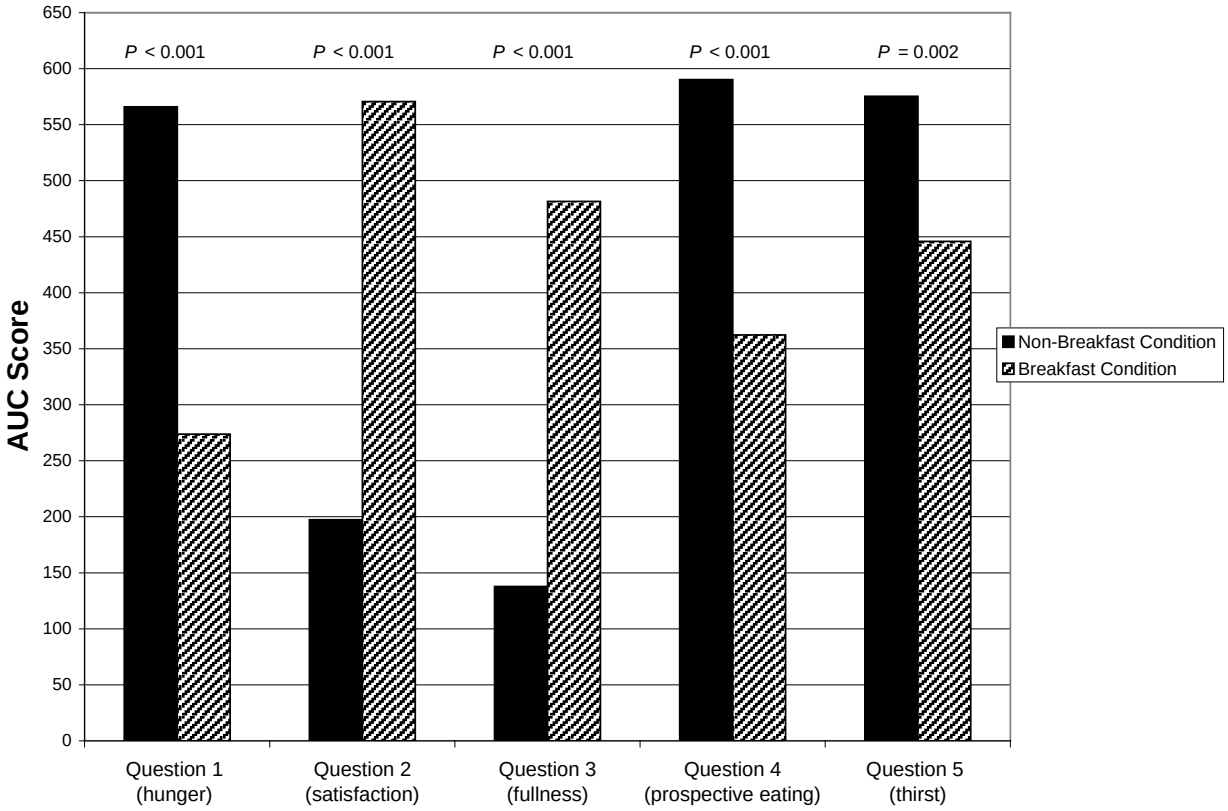


Figure 5. Comparison of area under the curve (AUC) for scores from subjective hunger and satiety questionnaires for breakfast and non-breakfast testing conditions

4.3.2 The Association Between Subjective Ratings of Hunger and Satiety and Plasma Acylated-Ghrelin and GLP-1 Concentrations Following Breakfast and Non-Breakfast Testing Conditions

Bivariate correlations were performed to investigate if there was a significant association between subjective ratings of hunger and satiety and plasma AG and GLP1 concentrations. Values were examined at each time point and for AUC. Because VAS scores were not normally distributed at several time points, Spearman correlations were mostly used for the analysis. The Spearman rank coefficient and the Pearson correlation coefficient (r) were not significantly

different from zero for all analyses, indicating that there was no relationship between subjective feelings of hunger and satiety and the physiological data. Data are presented in Table 9, and use of Spearman correlations is noted. The analysis was performed again with the previously mentioned outlier participants removed from the sample, and the significance of the correlations did not change.

4.3.3 Additional Correlational Analyses

Bivariate correlations were also performed to investigate if there was a significant association between hunger and energy intake. For each testing condition, mean VAS scores per question at the 120 minute time point and for AUC were compared to total energy intake, snack and discretionary intake, and discretionary intake. The Pearson correlation coefficient (*r*) was significantly different from zero for each analysis for the Breakfast condition only, except at the 120 minute time point for Question 4. However, at that that time point the Pearson correlation coefficient trended toward significance at each level of energy intake.

Because the assumption of normality was not met for energy intake data for the Non-Breakfast condition and for scores at the 120 minute time point of Question 2, Spearman rank correlation tests were performed for each of these analyses. At each level of energy intake, the Spearman rank coefficient was significantly different from zero for scores at the 120 minute time point of Question 2 for the Breakfast condition only. The Spearman rank coefficient was not significantly different from zero for all analyses for the Non-Breakfast condition at each level of energy intake. In addition, both Pearson correlation and Spearman rank coefficients were not significantly different from zero at the 120 minute time point or for AUC for each testing condition and at each level of energy intake for Question 5. Data are presented in Table 10.

Table 9. Correlation of subjective ratings of hunger and satiety and plasma acylated ghrelin and GLP-1 concentrations for breakfast and non-breakfast testing conditions (Spearman rank coefficient and Pearson correlation coefficient *(r)* reported)

		Acylated Ghrelin		GLP-1	
Subjective Rating (100 mm scale)	**Time Point**	Non-Breakfast Condition	Breakfast Condition	Non-Breakfast Condition	Breakfast Condition
Question 1: Overall, how hungry do you feel?	Baseline	.380[A]	-.092	.123	.121
	30 minute	.227	.272	-.026	.018
	60 minute	.230	-.046[A]	.260	.215
	120 minute	-.040	.116	.140	.171
	AUC[B]	.194	.123[A]	.255	.045
Question 2: Overall, how satisfied do you feel?	Baseline	-.403[A]	-.224[A]	-.140	-.223
	30 minute	-.366	-.301	-.404	-.008
	60 minute	-.122	-.063	-.242	-.255
	120 minute	-.142	-.164	-.315	-.117
	AUC	-.304	-.033[A]	-.290	.136
Question 3: Overall, how full do you feel?	Baseline	-.140	-.307	-.239	-.197
	30 minute	-.339	-.237	-.365	-.118
	60 minute	-.284	-.094	-.332	-.303
	120 minute	-.200	-.121	-.440	-.296
	AUC	-.275	-.099[A]	-.409	-.208
Question 4: Overall, how much do you think you could eat right now?	Baseline	.303[A]	-.114[A]	-.229	-.042
	30 minute	.308	.106[A]	.000	.251
	60 minute	-.143	-.181[A]	-.034	.277
	120 minute	-.131	-.062	.051	.054
	AUC	.005	-.084[A]	-.059	-.004
Question 5: Overall, how thirsty do you feel?	Baseline	.016[A]	-.242[A]	-.027	-.115
	30 minute	.127	.306[A]	-.213	-.408
	60 minute	-.064	.354[A]	-.192	-.387
	120 minute	-.033	.086	.021	-.199
	AUC	-.014	.242[A]	-.114	-.270

[A] Pearson correlation computed

[B] AUC = area under the curve for VAS scores from baseline through 120 minutes

Table 10. Correlation of subjective ratings of hunger and satiety and energy intake for breakfast and non-breakfast testing conditions (Pearson correlation coefficient and Spearman rank coefficient *(r)* reported)

		Total Daily Intake (kcals)		**Snack and Discretionary Intake (kcals)**		**Discretionary Intake (kcals)**	
Subjective Rating (100 mm scale)	**Time Point**	Non-Breakfast Condition Day[A]	Breakfast Condition Day	Non-Breakfast Condition Day[A]	Breakfast Condition Day	Non-Breakfast Condition Day[A]	Breakfast Condition Day
Question 1: Overall, how hungry do you feel?	120 min.	.278	.536*	.278	.539*	.336	.539*
	AUC[B]	-.138	.617*	-.138	.635*	-.062	.613*
Question 2: Overall, how satisfied do you feel?	120 min.[A]	-.174	-.422*	-.174	-.415*	-.209	-.407*
	AUC	-.160	-.640*	-.160	-.646*	-.207	-.637*
Question 3: Overall, how full do you feel?	120 min.	.061	-.406*	.061	-.410*	.020	-.402*
	AUC	.124	-.591*	.124	-.608*	.073	-.593*
Question 4: Overall, how much do you think you could eat right now?	120 min.	-.017	.375	-.017	.398	.044	.387
	AUC	-.271	.492*	-.271	.513*	-.207	.483*
Question 5: Overall, how thirsty do you feel?	120 min.	-.064	.013	-.064	-.019	-.066	.011
	AUC	-.085	-.110	-.085	-.136	-.085	-.103

*$P < 0.05$

[A] Spearman rank correlation computed.

[B] AUC = area under the curve for VAS scores from baseline through 120 minutes

5.0 DISCUSSION

5.1 INTRODUCTION

Two-thirds of adults in the United States are overweight or obese [1]. Increased rates of obesity are associated with increased rates of diabetes, cardiovascular disease and cancer, the leading causes of death in America [2-4]. With a decrease in the rates of overweight and obesity and the maintenance of a healthy body weight, it is likely that a decrease in the rates of these chronic diseases will follow. Regular breakfast consumption has been identified as a lifestyle behavior for weight loss maintenance, but the mechanism behind this association is unclear.

It is possible that eating breakfast initiates a hormonal response that impacts feelings of hunger and satiety, which in turn could lead to decreased energy intake later in the day. However, results of the current research on this association have been mixed, mainly due to differences in research methodology [8-11, 61, 62, 64]. Also, it is not clear if body weight plays a role in this relationship. If there is a relationship between breakfast consumption and daily energy intake, the mechanism linking the two needs to be clarified.

None of the studies reviewed examined within-subject differences in appetite-regulating hormones, energy intake, and feelings of hunger between breakfast consumption and non-breakfast conditions in normal weight, overweight, and obese sedentary women. Therefore, the purpose of the current study was to examine how eating breakfast acutely influenced plasma AG

and GLP-1 concentrations, subjective ratings of hunger and satiety, and daily energy intake in women compared to a day when breakfast was not eaten, and to explore the role of body weight on this influence.

5.2 SUMMARY OF MAJOR FINDINGS

5.2.1 The Effect of Breakfast on Acylated Ghrelin Concentrations

At baseline, plasma AG concentrations were not significantly different between breakfast and non-breakfast conditions. Following breakfast, AG concentrations significantly decreased from baseline levels to nadir levels within 60 minutes of eating and then approached baseline levels within an additional 60 minutes. Mean concentrations at the 30-minute and 60-minute time points were significantly lower than the baseline mean. After removing one outlier participant, mean concentrations at the 30- and 60-minute time points were also significantly lower than the mean at the 120-minute time point. Following the non-breakfast condition, concentrations slightly increased from baseline levels over the two-hour monitoring period, but not significantly. In addition, as hypothesized, concentrations at the 30-, 60-, and 120-minute time points following breakfast were significantly lower than those following the non-breakfast condition. These findings are similar to those of previous research done with normal weight and mildly overweight individuals [38, 57]. However, to the researchers' knowledge the current study is the only one to demonstrate the response of plasma AG following breakfast and non-breakfast conditions in normal weight, overweight, and obese sedentary women.

There was a large variability in fasting AG concentrations among reviewed studies [132-135], with one study reporting a mean fasting concentration of 51.7 pg/ml [135] and another reporting a mean fasting concentration of approximately 160.0 pg/ml [132]. These differences are likely due to differences in research methodology. For example, the former study included participants between the ages of 18 and 60, while the latter study included only healthy normal weight young women. Mean fasting AG concentrations in the current study (105.2 pg/ml) fell within the middle of the range of the reviewed studies.

To date, only two studies have examined plasma AG concentrations following breakfast and non-breakfast conditions. Liu et al. used a randomized crossover design similar to the current study in eight young, normal weight and mildly overweight men (mean age 24.5 ± 3.7 y, mean BMI 24 ± 2.1 kg/m^2) and reported a temporal profile of plasma AG concentrations following the breakfast condition that was identical to the profile in the current study. Following the non-breakfast condition, though, plasma AG concentrations remained steady in Liu's study, but at levels similar to those of nadir levels following the breakfast condition [38], while in the current study concentrations remained steady at levels similar to those of baseline following the breakfast condition. Therefore, Liu et al. observed significantly different concentrations between testing conditions at baseline but not at the other measured time points, while the current study observed the opposite. In addition, baseline concentrations of AG in the study by Liu et al. were approximately 70% lower on the breakfast testing day and approximately 85% lower on the non-breakfast testing day than concentrations in the current study. These differences could be due to the fact that sampling of blood began after 37.5 hours of a 61.5 hour fast in the study by Liu et al. while in the current study sampling began after a minimum of 12 hours of fasting. Other research has reported that total ghrelin levels significantly decrease with prolonged fasting [111],

but none of the reviewed studies examined the temporal profile of AG during a prolonged fast. It is possible that AG levels may also decrease to lower levels, similar to those of a fed-state, instead of continuing to increase when the physiological demands of the body can not be met with food for a prolonged period of time. Additional research investigating how AG changes over longer periods of time, perhaps up to 24 hours, in fed and fasting conditions could help to further explain the influence of AG on appetite regulation and energy intake.

The temporal profile of plasma AG concentrations in the current study was more closely related to the profile in the study by Lucidi et al [57]. In that randomized crossover study on six normal weight adults (male/female ratio 3/3, mean age 36 ± 2 y, mean BMI 23 ± .07 kg/m^2), concentrations decreased from baseline during the first 60 minutes of monitoring and then approached baseline levels within the next 60 minutes of monitoring as they did in the current study. Another similarity was that following the non-breakfast condition, concentrations remained steady at levels similar to those of baseline following the breakfast condition. However, in the study by Lucidi et al. baseline AG concentrations on each testing day were approximately 20% higher than baseline concentrations in the current study. Although men were included in the study by Lucidi et al., a gender effect is unlikely since other research has reported higher levels of AG in women than men by as much as 1.8-fold [136, 137]. The differences in AG concentrations could be due to differences in body weight between study participants. The current study included normal weight, overweight, and obese women while the study by Lucidi et al. included only normal weight men and women. Previous research has reported that total ghrelin concentrations are inversely correlated with BMI, with fasting levels being lower in obese individuals [49, 94, 102]. The influence of BMI on study outcomes is discussed further below. The findings of the current study demonstrate that breakfast consumption has a

significant effect on AG, an appetite-stimulating hormone. However, larger randomized crossover studies are needed to investigate differences in temporal patterns of AG concentrations following breakfast and non-breakfast conditions. Because many different factors, such as gender, age, and body weight, have been reported to affect AG, future studies need to control better for the influence of these factors in an effort to understand the role of AG in appetite regulation and energy intake.

5.2.2 The Effect of Breakfast on GLP-1 Concentrations

At baseline, plasma GLP-1 concentrations were not significantly different between breakfast and non-breakfast conditions. Following breakfast, GLP-1 concentrations increased from baseline levels to peak levels within 60 minutes of eating and then approached baseline levels within an additional 60 minutes. At no point were the differences between time points significant, even after removing the one outlier participant. Following the non-breakfast condition, concentrations slightly decreased from baseline levels over the two-hour monitoring period, but not significantly. However, as hypothesized, concentrations at the 30-, 60-, and 120-minute time points following breakfast were significantly greater than those following the non-breakfast condition.

As with fasting AG concentrations, there was some variability in fasting GLP-1 concentrations among reviewed studies [26, 138-141], with one study reporting a mean fasting concentration of 2.1 pm/L [26] and another reporting a mean fasting concentration of 4.6 pm/L [139]. These differences are likely due to differences in research methodology. The former study included only male participants between the ages of 18 and 26, while the latter study included male and female participants between the ages of 20 and 60. Mean baseline fasting

concentrations of GLP-1 in the current study (1.7 pm/L) were lower than the range of the reviewed studies. This may be due to a gender effect, as only women were included in the current study, while each of the reviewed studies included men.

None of the studies that were reviewed examined differences between endogenous GLP-1 concentrations following breakfast consumption and non-breakfast conditions in healthy adults. While many examined changes in GLP-1 concentrations following the manipulation of the content of a particular macronutrient in the breakfast meal [26, 51, 138-144], only one study examined changes in GLP-1 following a standard, mixed-nutrient breakfast. Unlike the current study, though, in that between-subjects study by Verdich et al. differences in the response of GLP-1 to breakfast were compared between normal weight (n = 12) and obese men (n = 19) before and after the obese men went through a weight loss program. Also, changes in GLP-1 following a non-breakfast condition were not part of the study design [84]. After eating breakfast, GLP-1 concentrations followed a similar temporal pattern as in the current study, increasing and peaking within 60 minutes and then approaching baseline levels within the next 60 minutes. This response occurred in normal weight and obese men, and after the obese men lost weight. However, because the aim of the study by Verdich et al. was to examine differences between the two groups before and after a weight loss program, the significance of within-subject differences in GLP-1 concentrations between time points was not discussed by the authors.

Another difference between the studies was that in the study by Verdich et al., GLP-1 concentrations were nearly 10 times higher than those in the current study at each measured time point. It is possible that this is due to a gender effect as Verdich et al. included only men in their study while the current study included only women. In a study by Carroll et al., men had

significantly higher levels of GLP-1 than women over a 60 minute monitoring period. In that study, though, concentrations were only approximately two times higher in men than women [145]. It is also possible that differences in outcomes were due to methodological differences in laboratory procedures. The study by Verdich et al. measured GLP-1 against standards of synthetic GLP-1 (7-36) amide using an antiserum that does not react with GLP-1-containing peptides from the pancreas. The current study measured GLP-1 using a standard kit. Results of GLP-1 concentration in the current study were similar to GLP-1 concentrations in other studies reviewed [37, 77, 83, 85]. The current study is the only one to examine the effect of breakfast consumption compared to a non-breakfast condition on the acute response of plasma GLP-1 concentrations in normal weight, overweight, and obese sedentary women, and the findings demonstrate that breakfast consumption has a significant effect on this appetite-suppressing hormone. However, larger studies of a similar design are needed to better understand the effect of breakfast on GLP-1. The influence of gender should also be considered when designing future studies.

5.2.3 The Effect of Breakfast on Daily Energy Intake

In the current study, daily energy intake was examined as: 1) total daily energy intake, which included breakfast and/or the snack provided by researchers plus all other discretionary intake recorded by the participants; 2) snack and discretionary intake, which included only the snack provided by researchers plus all other discretionary intake recorded by the participants; 3) snack intake only; and 4) discretionary intake only. It was hypothesized that daily energy intake would be significantly lower following breakfast consumption than following a non-breakfast

condition. However, no significant difference in energy intake between testing conditions was observed at any of the above energy intake levels.

Total daily energy intake was higher on the breakfast testing day than on the non-breakfast testing day by an average of 264 calories, but the difference was not significant. Even though overall intake was slightly higher on the breakfast testing day, on the non-breakfast testing day intake of the morning snack provided by the researchers was higher by an average of 27 calories and all other discretionary intake was higher by an average of 88 calories, but neither of these differences was significant. It appears that participants partially compensated for missing breakfast by eating more of the morning snack and by slightly increasing their discretionary intake on the non-breakfast testing day, but the increase in intake did not exceed the calories they missed by not eating breakfast. Therefore, a decrease in total daily energy intake did not follow breakfast consumption as hypothesized. This is puzzling since the effect of breakfast consumption on the response of AG and GLP-1 concentrations resulted in a pattern that was conducive to increased energy intake on the non-breakfast testing day. It is possible that even though significant changes in plasma AG and GLP-1 concentrations between testing conditions were observed, concentrations did not reach a threshold at which behavior is influenced to increase energy intake. If such a threshold exists, additional research involving endogenous concentrations of each hormone is warranted to explore possible associations with energy intake. It is also possible that even though AG and GLP-1 provided a physiological signal for hunger and satiety, that signal did not translate to a positive influence on increased energy intake.

The results of the current study are similar to those of recent research comparing energy intake on breakfast and non-breakfast days in children and adults [65, 146]. In a randomized

crossover trial that had a research design similar to the current study, Kral et al. showed that elementary school children (male/female ratio 6/15, mean age 9.2 ± 0.8 y, mean BMI-for-age percentile 57.3 ± 29.4) consumed an average of 362 more calories on the breakfast day than on the non-breakfast day, but the difference in that study was significant (P = 0.04) [146]. The metabolic differences between growing children and adults, though, complicate comparisons between the results of these studies. For example, it is possible that for children of the body weight in the Kral study, the physiological signals that influence increased energy intake are beneficial and needed for continued growth, while in adults other factors alter the signals that influence energy intake since continued growth is not needed.

Two other studies found similar results in adults. In a 10-day prospective analysis of food diaries kept by 280 obese (male/female ratio 75/205, mean age 45 ± 0.85 y, mean BMI 36.6 ± 0.2 kg/m^2) and 100 normal weight (male/female ratio 33/67, mean age 42 ± 0.2 y, mean BMI 32.5 ± 0.1 kg/m^2) participants, Schusdziarra et al. reported that obese participants consumed approximately 400 more calories and normal weight participants consumed approximately 500 more calories on days breakfast was eaten compared to days it was not eaten. Both differences were significant (P < 0.05) [65]. Similarly, Nicklas et al. reported that 504 young adults (mean age 23 y) consumed 568 more calories on days breakfast was eaten (P < 0.001) than on days breakfast was skipped [13]. However, neither of these studies involved a controlled, non-breakfast condition, therefore making comparison of results to the current study difficult. Regardless, all three studies demonstrated increases in total daily energy intake of various amounts on days when breakfast was eaten [13, 65, 146]. It is possible that the other studies reported greater energy intake amounts than the current study because they included male participants.

Only one study reported significantly lower intake following breakfast consumption. In a randomized crossover trial that involved breakfast consumption and non-breakfast conditions, participants in a study by Farshchi et al. consumed an average of 91 fewer calories on days when breakfast was eaten (P = 0.001) [21]. The design of that study, though, limited discretionary eating to four structured meal and snack times during both conditions, even though participants were allowed to eat whatever they chose. This may have limited the free-living conditions that were part of the design of the current study and the studies above and may have confounded its results.

Even though the increase in calories on the breakfast testing day in the current study was not clinically significant, it is significant in practice as an increase of approximately 100 calories per day every day could equate to a 10 pound weight gain over the course of a year. However, because snack and discretionary intake was slightly higher in the current study on days when breakfast was skipped, it might be advantageous for those trying to control their energy intake to eat a small breakfast daily. If calories at breakfast could be kept to a minimum and if eating breakfast could also help to limit energy intake at subsequent meals and snacks, it is possible that consuming a small breakfast daily could lead to a decrease in total daily energy intake that may have a significant beneficial impact on body weight over time.

Another factor to consider is that all of the participants in the current study stated that they ate breakfast at least four times per week. It is possible that having a regular breakfast eater skip breakfast for one day was not enough to invoke a response that may have led to an increase in total daily energy intake. Perhaps if the participants were followed for a week in which breakfast was skipped, an increase in total daily energy intake may have been observed. Conversely, an increase in total daily energy intake may have been observed if this same study

was conducted with participants who do not regularly eat breakfast. Additional research is warranted to investigate if either the amount of calories consumed at breakfast or if changing participants' established breakfast eating patterns for a longer period of time has a significant impact on total daily energy intake.

5.2.4 The Influence of BMI on Study Outcomes

Acylated Ghrelin (AG)

It was hypothesized that AG levels would be significantly higher in overweight participants than in normal weight participants and in obese participants than in overweight participants for two hours following breakfast consumption than for two hours following the non-breakfast condition. No interaction effect was observed, though. Because ghrelin has been reported to play a role in meal initiation, with plasma concentrations increasing to the point of spontaneous feeding [22, 54, 58], it may seem reasonable to conclude that plasma AG concentrations would be higher in individuals in positive energy balance, such as those who are overweight or obese. A relationship has been demonstrated with infusion studies in which energy intake levels increased following subcutaneous administration of AG prior to meal consumption [69, 70]. In contrast to the above conclusion, though, other research has reported total plasma ghrelin levels to be negatively correlated with BMI [49, 94, 102], with fasting levels lower and postprandial decreases attenuated in obese individuals when compared with normal weight individuals [49, 101-104]. These differences have been observed to improve, though, with weight loss [27]. Therefore, it was proposed that total plasma ghrelin concentrations inversely change with body weight to act as part of a negative feedback mechanism to maintain energy homeostasis, with up-

regulation occurring under conditions of negative energy balance and down-regulation occurring under conditions of positive energy balance [101].

With respect to AG, though, only one study has examined the association between plasma AG concentrations and body weight in healthy adults and, as in the current study, found no interaction effect [97]. However, the lack of a correlation may be due to the small sample sizes in these studies (n = 19 and n = 18, respectively). Larger randomized crossover trials are needed to investigate if body weight is associated with the effects of AG on energy intake. It is also possible that no association was observed in the studies examining AG and body weight while an association was observed in studies examining total plasma ghrelin and body weight because of the presence of des-acyl ghrelin. As reviewed above, the ratio of des-acyl ghrelin to acylated ghrelin has been reported to change even though total plasma ghrelin levels do not change [56]. The significant association reported in the studies examining total plasma ghrelin may be due to an effect by des-acyl ghrelin. It is, therefore, important for future studies to assess changes in acylated and des-acyl ghrelin simultaneously when examining associations between ghrelin concentrations and body weight.

GLP-1

It was hypothesized that GLP-1 levels would be significantly lower in overweight participants than in normal weight participants and in obese participants than in overweight participants for two hours following breakfast consumption than for two hours following the non-breakfast condition. In the final analysis, a significant interaction effect was observed. Following breakfast consumption, plasma GLP-1 concentrations were significantly lower in overweight and obese participants than in normal weight participants. This conclusion would seem reasonable

since GLP-1 plays a role in satiety and individuals in positive energy balance, such as those who are overweight or obese, would be expected to have lower feelings of fullness after meal consumption than those in energy balance, such as normal weight individuals. Other research has supported this conclusion and has also reported an increase in GLP-1 concentrations with weight loss [84]. Although GLP-1 is only one of several anorexigenic hormones, the association observed between body weight and GLP-1 in the current study supports the importance of its role in energy balance.

Daily Energy Intake

Although it was not a specific aim of the study, the association between body weight and daily energy intake was explored. In the final analysis, a significant interaction effect was observed between normal weight and overweight participants, and a weak interaction effect was observed between overweight and obese participants, but no interaction effect was observed between normal weight and obese participants. This seems counterintuitive. However, daily energy intake increased following breakfast consumption when compared to the non-breakfast condition for overweight participants while it decreased for normal weight and obese participants. It is possible that with a larger sample size these results would not be repeated. Although, if the outcomes were the same, it could be due to a BMI threshold being reached at which the response to the physiological stimulation of appetite is blunted. As reviewed above, the ghrelin system is impaired with weight gain. It is possible that a decrease in sensitivity to the ghrelin response is not evident until an individual reaches a certain body weight. If so, even when preprandial AG levels are lower than those of normal weight individuals, overweight individuals may still respond by increasing energy intake. The decrease in sensitivity to the ghrelin response may not

be evident at preprandial AG levels higher than those of obese individuals. It is also possible that obese individuals underreported daily intake amounts, therefore resulting in lower daily energy intake following breakfast than overweight participants. Research has reported that obesity is significantly associated with underreporting of energy intake [147-150]. Larger crossover trials are needed to further elucidate any possible associations between BMI and energy intake following breakfast consumption and non-breakfast conditions.

5.2.5 The Effect of Breakfast on Subjective Ratings of Hunger and Satiety

Subjective ratings of hunger and satiety were measured using 100 mm visual analog scales (VAS). As hypothesized, subjective ratings of hunger were significantly lower and subjective ratings of satiety were significantly higher during the two-hour monitoring period following breakfast consumption than following the non-breakfast condition. For each question, ratings were not significantly different between the two testing conditions at baseline, but they were at all other time points. In addition, following breakfast consumption, although the mean rating of hunger at 120 minutes was significantly lower than at baseline, it was significantly higher than at 30 minutes. Also, on one of the two questions assessing satiety, the mean rating at 120 minutes was significantly lower than at 30 minutes, even though it was still significantly higher than at baseline. This suggests that the effect of breakfast consumption on subjective feelings of hunger and satiety begins to diminish within two hours of eating breakfast. By comparison, following the non-breakfast condition hunger ratings trended toward being significantly higher and satiety ratings trended toward being significantly lower over the two-hour monitoring period, suggesting that feelings of hunger increase in the short term when breakfast is skipped.

While numerous other studies examined changes in subjective ratings of hunger and satiety following the manipulation of macronutrients in a breakfast meal, the previously reviewed study by Farshchi et al. was the only study from the reviewed literature to examine differences in hunger and satiety ratings between breakfast consumption and non-breakfast conditions. However, the design of Farshchi's study was to examine changes in subjective ratings following a two-week period of daily breakfast consumption compared to a two-week period of daily breakfast omission. At each assessment visit participants were fed breakfast prior to completing VAS questionnaires in order to assess the impact of testing conditions on how hungry participants felt after eating breakfast [21]. Unfortunately, with respect to measuring subjective ratings of appetite, a non-breakfast condition was not part of the study design. However, the questions assessing hunger and satiety in that study were the same as the questions in the current study. Comparing the responses between the two studies, following breakfast consumption the temporal patterns of the responses to each question were similar in both studies, adding support to the conclusion of the current study that the effect of breakfast consumption on subjective feelings of hunger and satiety begins to diminish within two hours of eating breakfast. To the knowledge of the researchers of the current study, ours is the only one to examine differences in subjective ratings of hunger and satiety following breakfast consumption and non-breakfast conditions.

5.2.6 The Association between Subjective Ratings of Hunger and Satiety and Study Outcomes

To test the strength of the relationships between subjective feelings of hunger and the other dependent variables, VAS scores were correlated with AG concentrations, GLP-1

concentrations, and daily energy intake. With respect to the appetite-regulating hormones, it was hypothesized that subjective ratings of hunger would positively correlate with plasma AG concentrations and subjective ratings of satiety would positively correlate with plasma GLP-1 concentrations for the two-hour monitoring period following breakfast consumption and a non-breakfast conditions. Surprisingly, this did not occur. At no point on either testing day did the VAS scores for any question significantly correlate with concentrations of either of the appetite-regulating hormones.

Although conclusions from the reviewed literature are mixed regarding a relationship, there is support for this lack of correlation. Erdmann et al. concluded in several studies that ghrelin concentrations did not correlate with feelings of hunger and satiety following carbohydrate-, protein-, or fat-rich breakfast meals [30, 31, 33]. These studies only looked at total plasma ghrelin concentrations, though, so it is difficult to compare results with the current study. Blom et al. found a significant relationship between total plasma ghrelin concentrations and VAS scores for hunger and satiety following breakfast consumption in one study [25], but in another study that examined AG and GLP-1 concentrations following a standard mixed breakfast and a high-protein breakfast, a significant relationship was not observed for either condition [26]. None of the other reviewed studies correlated AG concentrations with VAS scores.

With respect to GLP-1, most studies only found a significant relationship with subjective ratings of hunger and satiety when exogenous GLP-1 was infused. For example, in a meta-analysis of nine GLP-1 infusion studies, Verdich et al. reported that differences in plasma GLP-1 concentrations significantly correlated with differences in subjective ratings of fullness, but not with ratings of hunger [52]. However in the previously mentioned study by Verdich et al. that examined changes in endogenous GLP-1 concentrations after a weight loss intervention, only a

weak correlation between GLP-1 AUC and the AUC of subjective appetite ratings was observed [84]. Comparing results from studies examining supraphysiological infused doses and regular endogenous concentrations of GLP-1, Verdich et al. concluded that endogenous plasma GLP-1 concentrations may not be enough to stimulate a subjective satiety response [84].

The discrepancy in the significance of results from the latter study by Verdich et al. and the current study may be due to the fact that the monitoring of the dependent variables in the Verdich study lasted 180 minutes, while in the current study monitoring lasted only 120 minutes. Adding to this disparity, in a study by Adam et al. in which monitoring also lasted 120 minutes, a significant correlation was observed between increased postprandial concentrations of GLP-1 and subjective ratings of satiety at 60 and 90 minutes following consumption of a breakfast meal with a preload of water. The correlation was not significant, though, following a breakfast meal with a preload of a galactose and guar gum beverage [85], which suggests that a macronutrient component may mediate the association. Given the monitoring times in the above studies [84, 85], though, it is also possible that a significant relationship between changes in GLP-1 and subjective ratings of hunger and satiety is only realized over longer periods of time. Lastly, it is also possible that since GLP-1 is only one of several anorexigenic peptides, other hormones may play a role in the relationship that confound the correlation between GLP-1 and subjective ratings of hunger and satiety. Although four of the above studies examined other anorexigenic hormones along with GLP-1, none reported that the other hormones were included as covariates when analyzing the relationship between GLP-1 concentrations and VAS scores [26, 42, 75, 84].

Although not a specific aim of the study, VAS scores were correlated with total daily energy intake, snack and discretionary intake, and discretionary intake only. Following breakfast consumption, daily intake at each intake level significantly correlated with subjective ratings of

hunger and satiety. However, there was not a significant relationship with either hunger or satiety following the non-breakfast condition. Only one of the reviewed studies examined the relationship between subjective ratings of hunger and satiety and energy intake and found no correlation between the difference in ad libitum energy intake and difference in both fullness and hunger ratings [52]. In the current study, it is interesting that a significant correlation was observed following breakfast consumption but not following the non-breakfast condition. This could suggest that in a fed state participants were more perceptive of the physiological drive and better able to identify feelings of hunger and satiety with corresponding changes in AG and GLP-1 than they were in a fasted state. Subjective feelings of hunger and satiety may be interpreted better when AG and GLP-1 are trending toward levels of a fed state. If accurate, this may explain how breakfast consumption influences physiological mechanisms that contribute to hunger, satiety, and eating behavior.

5.3 LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Only sedentary but otherwise healthy women between the ages of 18 and 40 were included in the current study. Caution should be taken before generalizing the results of this study to other individuals. In addition, the following are limitations of the current study that may have contributed to the observed findings:

1. Although it was determined that 19 participants were needed in order to detect a moderate to large effect size in differences of studied outcomes, only 18 participants were included in most analyses. Due to missing data or values outside of the normal range, some of the analyses included as few as 14 participants. While including 18 participants

is still enough to detect a large effect size with the statistical analyses used, analyses performed with fewer than 15 participants would not be powered to detect significant differences in outcomes. Also, this study was underpowered to examine differences in AG and GLP-1 concentrations and energy intake between BMI groups. A significant Condition × Time × BMI interaction was observed with GLP-1 concentrations and a Condition × BMI interaction was observed with daily energy intake. Although these findings suggest that body weight may influence appetite regulation and energy intake, additional, properly powered studies should be conducted to fully examine any associations.

2. As the previously reviewed research has observed, the macronutrient content of the breakfast meal may influence AG and GLP-1 concentrations as well as subjective ratings of hunger and satiety. In the current study, a standardized, mixed meal was used that provided commonly proportioned amounts of carbohydrate, protein, and fat in an effort to control for this influence. In the final analysis, though, daily energy intake did not significantly change following breakfast consumption when compared to a non-breakfast condition. Because of the conflicting results in research examining the impact of macronutrients on ghrelin and GLP-1 responses and subjective ratings of hunger and satiety, additional research is needed that includes a non-breakfast condition as part of its study design. If the role of breakfast in energy balance is beneficially influenced by a particular macronutrient, identifying the macronutrient and exploring the physiological mechanism of its action could be advantageous to those trying to achieve a healthy body weight.

3. Only AG and GLP-1 were examined in this study because both are gut-based hormones that are particularly responsive to food intake. However, as previously reviewed, they are only two of many appetite-regulating polypeptides. Other orexigenic peptides, including neuropeptide Y and agouti-related protein, and anorexigenic peptides, including leptin, cholecystokinin, and peptide YY, all play a role in appetite regulation and often work in tandem, sometimes influencing or inhibiting the secretion of one another. In addition, changes in body weight influence concentrations of many of these peptides. Therefore, it is quite possible that the findings of the current study were influenced by changes in these other appetite-regulating polypeptides.
4. Although it was hypothesized in the current study that total daily energy intake would be significantly lower following breakfast consumption than following a non-breakfast condition, energy intake was not significantly different between conditions. It is possible that differences in subsequent energy intake may be observed following meal consumption and non-meal conditions involving lunch or dinner. Research of a design similar to the current study should be conducted on lunch and dinner consumption to explore possible effects they may have on subsequent energy intake.
5. As with other research examining energy intake in free-living situations, energy intake data relied on self-report by the participants. Under- and over-reporting of energy intake is common when research participants are asked to be conscious of the food choices they make. Because analyses of energy intake were of a within-subjects design, and because the specific hypotheses of the study were not shared with the participants, the potential error associated with self-reported energy intake may have been reduced.

6. Blood samples were taken via a needle stick at four time points for each testing condition. With four participants, multiple attempts were required which slightly delayed the timing of the blood draws. Although the delay in obtaining the blood samples should not affect the findings of the current study, it is a possibility. Future studies should consider using an angiocatheter to reduce the difficulty in drawing blood and to improve the accuracy of timed blood draws.

5.4 CONCLUSIONS

Breakfast consumption has been identified as a strategy for achieving energy balance and maintaining weight loss, but mechanisms to explain its effects have not been fully explored. Results from research investigating the association between breakfast consumption and energy balance have been conflicting, mainly due to differences in research design. Findings from the current study indicate that even though a significant acute hormonal response was observed following breakfast consumption when compared to a non-breakfast condition, total daily energy intake between conditions was not significantly different. However, observed, statistically insignificant changes in energy intake following breakfast consumption could translate into energy balance over time. In addition, findings from our study suggest that body weight may play a role in the pathway by which breakfast consumption may impact energy intake. Finally, subjective feelings of hunger significantly correlated with energy intake following breakfast consumption but not following the non-breakfast condition, suggesting that perceptions of the physiological drivers of appetite may be more sensitive following breakfast consumption than

when breakfast is skipped. Additional studies with a larger sample size should be conducted to further investigate these associations.

APPENDIX A

INFORMED CONSENT DOCUMENT

University of Pittsburgh

School of Education
Physical Activity and Weight Management Research Center

Suite 600 Birmingham Towers
2100 Wharton Street
Pittsburgh, PA 15203
412-488-4184
Fax: 412-488-4174

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: The Effect of Breakfast Consumption on the Acute Response of Plasma Acylated-Ghrelin and Glucagon-Like Peptide 1 Concentrations in Adult Women

PRINCIPAL INVESTIGATOR:
Thomas A. Hritz, M.S., R.D., L.D.N.
Physical Activity and Weight Management
Research Center
University of Pittsburgh
Birmingham Towers, Suite 600
2100 Wharton St.
Pittsburgh, PA 15203
Telephone: 412-488-4184

CO-INVESTIGATORS:
John M. Jakicic, Ph.D.
Department of Health and Physical Activity
University of Pittsburgh
Birmingham Towers, Suite 600
2100 Wharton Street
Pittsburgh, PA 15203
Telephone: 412-488-4184

Amy D. Otto, Ph.D., R.D., L.D.N.
Department of Health and Physical Activity
University of Pittsburgh
Birmingham Towers, Suite 600
2100 Wharton Street
Pittsburgh, PA 15203
Telephone: 412-488-4184

Diane Helsel, Ph.D., R.D., L.D.N., C.S.S.D.
Assistant Professor
Department of Sports Medicine & Nutrition
University of Pittsburgh
4051 Forbes Tower
Pittsburgh, PA 15213
Telephone: 412-383-6532

Rhobert W. Evans, Ph.D.
Assistant Professor
Department of Epidemiology
University of Pittsburgh
502 Parran Hall
Pittsburgh, PA 15261
Telephone: 412-624-2020

SOURCE OF SUPPORT: School of Education, University of Pittsburgh

DESCRIPTION:

Excess body weight is associated with increased risk for developing heart disease, diabetes, and some forms of cancer. Maintaining a healthy body weight can improve the risk for these conditions. Once a healthy body weight is achieved, the goal in maintaining it is to match calories eaten to calories burned and to be in a state of energy balance. Research has identified regular breakfast consumption as a strategy to achieve energy balance. However, it is not fully understood how this occurs. The purpose of this study is to examine the effect of breakfast consumption on hormones that have been shown to affect appetite. It will also examine the effect of breakfast on feelings of hunger and fullness and on how much you eat the rest of the day.

You are being invited to take part in this research study because you are a woman who regularly eats breakfast and does not engage in regular physical activity. People invited to participate in this study are women between 18-40 years of age. The study is being performed on a total of 21 individuals and will be conducted at a University of Pittsburgh facility.

If you decide to take part in this research study, you will be asked to attend one orientation session and two testing sessions. At the orientation session, details of the study will be reviewed and an opportunity to have questions answered will be provided. In addition, you will be asked to complete some questionnaires and undergo some screening and experimental procedures if you are considered eligible to participate. The orientation session will last approximately 60 minutes.

The following procedures that are not part of your standard medical care will be conducted at the orientation session and the testing sessions:

Screening Procedures:

Procedures to determine if you are eligible to take part in a research study are called "screening procedures". For this research study, the screening procedures include:

Completion of a questionnaire about eating behaviors (Three-Factor Eating Questionnaire) and a detailed medical history. These will take approximately 30 minutes to complete and will allow the investigators to determine if you have any significant medical conditions that may affect the outcomes of this study.

Pregnant women, women who have been pregnant within nine weeks of starting the study, or women who are currently breast-feeding an infant will not be allowed to take part in this study. A urine pregnancy test will be used to determine pregnancy status at each testing session prior to its start.

Experimental Procedures:

If you qualify to take part in this research study, you will be asked to undergo assessments of height, weight, waist and hip measurements, body composition, and resting energy expenditure. These assessments will take place at the Physical Activity and Weight Management Research Center in Birmingham Towers (South Side of Pittsburgh) at the University of Pittsburgh. These assessments will be completed in approximately

90 minutes. A brief explanation of each follows:

A. Body Weight and Height (5 minutes): Your body weight will be measured using a standard medical scale. Your height will be measured with a ruler that is attached to a flat wall. These will be measured at the orientation visit and prior to the two testing sessions.

B. Waist and Hip Measurements (5 minutes): Your waist and hips will be measured by an investigator using a standard tape measure. They will be measured at the orientation visit and prior to the two testing sessions.

C. Body Composition (20 minutes): Your body composition is the amount of fat weight and lean weight (muscle and bone) that you have on your body. Your body composition will be measured using a technique known as Bioelectric Impedance Analysis (BIA). This procedure requires that a small electrode be placed on your hand, wrist, ankle, and foot. A low-level signal that is not harmful to you and that you will not feel is transmitted between the electrodes. BIA will be measured at the orientation visit and prior to the two testing sessions.

D. Resting Energy Expenditure (50 minutes): Your resting energy expenditure (REE), or the number of calories that you burn at rest, will be measured on each of the testing days. For this procedure, you will be asked to lie quietly in a room for approximately 30 minutes to ensure that your body is in a rested state. Then, a plastic canopy will be placed over your upper body, and the air that you breathe in and out will be analyzed for a period of 20 minutes. REE will only be measured prior to the two testing sessions.

If your are currently a subject in another ongoing research study at the Physical Activity and Weight Management Research Center and you have already completed assessments of height, weight, waist and hip measurements, body composition, and resting energy expenditure within 4 weeks of signing this consent document, and if those tests utilized the same procedures as described above, you grant the investigators permission to use the results from the previously completed tests so that you do not need to perform these tests again for this current study.

Breakfast Consumption and Non-Breakfast Sessions:

At an orientation meeting, you will be asked to schedule two visits to the Physical Activity and Weight Management Research Center. One visit will require you to consume a breakfast provided by the investigators and the other will require that you do not eat breakfast. These visits will be separated by at least 3 days and be within days 7 and 21 of your menstrual cycle. The order in which these visits take place will be randomly determined using a method similar to flipping a coin. A more detailed description of each of these visits is shown below.

A. Breakfast Consumption Condition: On this day of testing you will report to the research center between the hours of 7:00 a.m. and 9:00 a.m. having fasted for 12 hours. You should eat no food during the 12 hour fasting period, but drinking water is permitted. All testing procedures will be reviewed and the following will occur over a total of approximately 4 hours:
   a. The above experimental procedures (height, weight, waist and hip measurements, body composition, and resting energy expenditure) will be performed.
   b. You will be asked to complete a questionnaire related to your feelings of hunger and fullness

immediately prior to eating breakfast and at 30, 60, and 120 minutes after finishing breakfast.

c. Blood will be taken 4 times throughout the course of the testing session via a needle stick. A sample will be taken immediately prior to eating breakfast and at 30, 60, and 120 minutes after finishing breakfast. Each blood sample will be approximately 1 tablespoon of blood, with a total of approximately 4 tablespoons of blood being collected during this entire session. Your blood will be analyzed to measure levels of acylated-ghrelin and glucagon-like peptide 1 (GLP-1) which are biomarkers of metabolism and digestion that are thought to be involved in weight control. Serum insulin and glucose will also be analyzed. Blood samples will be obtained by a trained phlebotomist or medical technician. In addition, at the time of each blood draw, your blood glucose level will be measured using a standard glucometer to ensure that your blood glucose does not drop too low. If it does, the test will be terminated.

d. You will be provided with a choice of breakfast meals. Each will include the same amount of calories and provide 20% of your estimated daily needs based upon your height, weight, and age. You will be required to completely eat the breakfast meal within 15 minutes. You will be able to choose from either a Luna Bar and whole milk or a toasted English muffin, cheddar cheese, and apple juice as the breakfast meal. You will be able to choose from one of several flavors of Luna Bars. If you are lactose intolerant you will be provided with Lactaid milk.

e. Upon completion of breakfast you will be brought to a separate room in which you will be asked to rest quietly in a seated position for 2 hours. During this time, you will have access to newspapers, magazines, books, and a video that you can watch. The additional blood samples will be collected and the additional questionnaires will be completed during this time as detailed above at 30, 60, and 120 minutes following breakfast.

f. Prior to leaving the research center, you will be required to consume a small snack and water.

g. To keep track of the foods you ate and the physical activity you engaged in on the day of testing, you will be required to document all foods consumed and all physical activity in a food and physical activity diary that you will be provided with. Prior to leaving the research center, a staff member will assist you in recording the food eaten during the testing session in the diary. The staff member then will remind you to record all foods eaten and all physical activity for the rest of the day in the food and physical activity diary and will answer any questions you have. On the day after your testing session, a staff member will call you to review the entries in your diary. You will be asked to schedule a time for the staff member to call you and provide him/her with a phone number at which you can be reached prior to leaving the research center.

B. Non-Breakfast Condition: On this day of testing you will report to the research center between the hours of 7:00 a.m. and 9:00 a.m. having fasted for 12 hours. You should eat no food during the 12 hour fasting period, but drinking water is permitted. All testing procedures will be reviewed and the following will occur over a total of approximately 3 hours:

a. The above experimental procedures (height, weight, waist and hip measurements, body composition, and resting energy expenditure) will be performed.

b. You will be asked to complete a questionnaire related to your feelings of hunger and fullness immediately after the procedures are reviewed with you and at 30, 60, and 120 minutes after a 15-minute waiting period during which you will sit quietly without eating.

c. Blood will be taken 4 times throughout the course of the testing session via a needle stick. A sample will be taken immediately after the procedures are reviewed with you and at 30, 60, and 120 minutes after a 15-minute waiting period during which you will sit quietly without eating. Each blood sample

will be approximately 1 tablespoon of blood, with a total of approximately 4 tablespoons of blood being collected during this entire session. Your blood will be analyzed to measure levels of acylated-ghrelin and GLP-1 which are biomarkers of metabolism and digestion that are thought to be involved in weight control. Serum insulin and glucose will also be analyzed. Blood samples will be obtained by a trained phlebotomist or medical technician. In addition, at the time of each blood draw, your blood glucose level will be measured using a standard glucometer to ensure that your blood glucose does not drop too low. If it does, the test will be terminated.

d. You then will be brought to a separate room in which you will be asked to rest quietly in a seated position for 2 hours. During this time, you will have access to newspapers, magazines, books, and a video that you can watch. The additional blood samples will be collected and the additional questionnaires will be completed during this time as detailed above at 30, 60, and 120 minutes following the 15-minute waiting period.
e. Prior to leaving the research center, you will be required to consume a small snack and water.
f. To keep track of the foods you ate and the physical activity you engaged in on the day of testing, you will be required to document all foods consumed and all physical activity in a food and physical activity diary that you will be provided with. Prior to leaving the research center, a staff member will assist you in recording the food eaten during the testing session in the diary. The staff member then will remind you to record all foods eaten and all physical activity for the rest of the day in the food and physical activity diary and will answer any questions you have. On the day after your testing session, a staff member will call you to review the entries in your diary. You will be asked to schedule a time for the staff member to call you and provide him/her with a phone number at which you can be reached prior to leaving the research center.

RISKS and BENEFITS:

The possible risks of this research study may be due to the foods you are asked to eat and the assessments that will be performed.

Risks

A. Risk of Bioelectrical Impedance Analysis (BIA): You may experience skin irritation or skin redness from electrodes being placed on your skin. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people).

B. Risk of Measuring Resting Energy Expenditure: The measurement of resting energy expenditure will be done using a ventilated hood. The only adverse factor associated with this is that you may experience a feeling of claustrophobia. The risk of this happening to you is infrequent because it occurs in less than 1-10% of people (1 to 10 out of 100 people). A person will be bedside at all times and check to see if you are comfortable. The transparent hood can be easily removed if necessary.

C. Risk Associated with Completion of Questionnaires: You may experience non-physical risks such as boredom, frustration, stress, and time constraints when completing the questionnaires. The risk of this happening to you is likely because this occurs in more than 25% of people (more than 25 out of 100 people). You may also experience the rare occurrence of breach of confidentiality with regard to information provided by you on the questionnaires. The risk of this happening is low because it occurs

in less than 1% of people (less than 1 out of 100 people).

D. Risk of Drawing Blood: The risks of drawing blood from a vein include discomfort at the site of puncture and possible bruising and swelling around the puncture site. The risks of this happening to you are likely because they occur in more than 25% of people (more than 25 out of 100 people). Although rare, it is possible that you may develop an infection or experience faintness from the procedure. The risk of an infection or fainting occurring is rare because they occur in less than 1% of people (less than 1 out of 100 people). You may also experience the rare occurrence of breach of confidentiality with regard to stored blood samples. The risk of this happening is low because it occurs in less than 1% of people (less than 1 out of 100 people).

E. Risk of Consuming a Solid or Liquid Meal Replacement: Among the options for the breakfast meal and snacks provided for you to consume during the testing sessions are meal replacement bars and shakes. There is a possibility that you may not like the taste of the meal replacement. Also, consuming some of them may result in bloating, gas, and indigestion. This is rare and occurs in less than 1% of people (less than 1 out of 100 people).

Benefits

There are no direct benefits that you will receive from participating in this study. However, knowledge gained from this study may provide a future benefit, for example, to those who may want to lose or maintain weight.

NEW INFORMATION:

You will be promptly notified if any new information develops during the conduct of this research study which may cause you to change your mind about continuing to participate.

COSTS and PAYMENTS:

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. These costs will be paid by the sponsor of this research study.

You will be paid $300 upon completion of all testing procedures which include an orientation session, the breakfast consumption session, and the non-breakfast session described above. You will be paid $100 after you complete the first testing session, $100 after you complete the second testing session and an additional $100 if you complete both testing sessions and follow-up phone interviews the day after each testing session. Thus, a total of $300 can be earned for your complete participation in the study.

COMPENSATION FOR INJURY:

University of Pittsburgh researchers and their associates who provide services at the University of Pittsburgh Medical Center (UPMC) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that the research procedures have resulted in an injury to you, immediately contact the

Principal Investigator who is listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

CONFIDENTIALITY:

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. If you should withdraw from participation in this study prior to its completion, any collected data and any biological specimens collected will be de-identified and rendered anonymous. They will be retained in this format and any identifiable data will be destroyed at this point. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Blood Samples

At this point, funding is only available to analyze your blood samples for acylated-ghrelin, GLP-1, insulin and glucose. However, stored samples of your blood will be used to examine other biomarkers that may affect hunger and satiety as additional funding becomes available. Therefore, obtained blood samples will be stored indefinitely in a locked freezer in the Physical Activity and Weight Management Research Center. Your identity on these blood samples will be indicated by a case number rather than by your name. The information linking the case number to your name will be stored separately in a secure location. Samples of your blood may be shared with secondary investigators. However, the samples will only be identified by case number and not by your name.

Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an

order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

If we should find out about a medical condition you were unaware of, with your written permission, this information will be shared with the doctor of your choice.

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of six years after final reporting or publication of a project.

RIGHT TO PARTICIPATE or WITHDRAW FROM PARTICIPATION:

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above. Any collected data and any biological specimens collected will be de-identified and rendered anonymous. They will be retained in this format and any identifiable data will be destroyed at this point.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

It is possible that you may be removed from the research study by the researchers if, for example, your health status changes and it does not appear that is safe for you to reduce your food intake or if you should become pregnant during the study

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations that have occurred during my participation.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____	_____	_____
Participant's Signature	Printed Name of Participant	Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____	_____
Printed Name of Person Obtaining Consent	Role in Research Study
_____	_____
Signature of Person Obtaining Consent	Date

APPENDIX B

BASELINE VISIT DATA COLLECTION FORM

Subject ID_____ Date consent signed_____

Baseline Study Procedures	Performed per Protocol and Documented?	Y/N
Telephone screening	Date_____ By _____	
Orientation session	Date_____ By _____	
Eating Inventory questionnaire completed		
Medical history questionnaire completed		
Height, Weight, BMI	Hgt_____cm. Wgt_____lbs. BMI_____kg/m^2	
Waist/Hip measurements	Waist (umbilicus)_____cm _____cm _____cm Waist (iliac crest)_____cm _____cm _____cm Hips_____cm _____cm _____cm	
Bioelectric Impedance Analysis (BIA)	Resistance_____ohms Reactance_____ohms	
Schedule Visit 1 and Visit 2 within 7-21 days of onset of menstrual cycle	Date of LMP_____ Date of Visit 1_____ Date of Visit 2_____ Randomized to: ❑ Breakfast ❑ Non-breakfast	
Selection for breakfast visit	❑ Luna bar & milk ❑ English muffin, cheese, apple juice	
Prep for next visit: • Fast for at least 12 hours • Abstain from vigorous PA day before visit • Avoid OTC meds. day before visit • Arrive to visit by vehicle	Reviewed with subject? Comments:	

Signature_____ Date_____

APPENDIX C

NON-BREAKFAST CONDITION DATA COLLECTION FORM

Subject ID_____ Acrostic_____ Date consent signed_____

Non-Breakfast Testing Session Date:_____	**Performed per Protocol and Documented?**	**Y/N**
Confirm: • Fasted for at least 12 hours • Abstained from vigorous PA day before visit • Avoided OTC meds. day before visit • Arrived to visit by vehicle		
Urine pregnancy test	❑Negative❑Positive (if positive, stop testing)	
Resting Energy Expenditure	Time done:_____ Rested for 30 mins. in supine position in dark room Start time_____ End time_____ Steady state measurement under canopy (complete test after REE stable for 5 minutes) Start time_____ End time_____	
Height, Weight, BMI	Hgt_____cm. Wgt_____lbs. BMI_____kg/m^2	
Waist/Hip measurements	Waist (umbilicus)_____cm _____cm _____cm Waist (iliac crest)_____cm _____cm _____cm Hips_____cm _____cm _____cm	
Bioelectric Impedance Analysis (BIA)	Resistance_____ohms Reactance_____ohms	
Baseline blood draw & hunger/satiety questionnaire	Blood sample: Questionnaire: Baseline_____ _____ By_____	
Waiting period	Start time_____ End time_____	
Rested quietly for next 2 hours with standardized video and periodicals Blood draw and hunger/satiety questionnaire completion at 30, 60 and 120 minutes following completion of waiting period	Resting start_____ End_____ Blood samples: Questionnaire: 30 mins._____ _____ 60 mins._____ _____ 120 mins._____ _____ By_____	
Completion: • Snack prior to leaving • Record food eaten in food & PA diary • Schedule time to call & review diary entries • Confirm next testing visit date (if applicable)	Snack choice: ❑Luna Bar & H_2O ❑Slim Fast Shake & H_2O Scheduled phone call time:_____	

Signature_____ Date_____

APPENDIX D

BREAKFAST CONDITION DATA COLLECTION FORM

Subject ID_____ Acrostic_____ Date consent signed_____

Breakfast Testing Session **Date:_____**	**Performed per Protocol and Documented?**	**Y/N**
Confirm: • Fasted for at least 12 hours • Abstained from vigorous PA day before visit • Avoided OTC meds. day before visit • Arrived to visit by vehicle		
Urine pregnancy test	❑Negative ❑Positive (if positive, stop testing)	
Resting Energy Expenditure	Time done:_____ Rested for 30 mins. in supine position in dark room Start time_____ End time_____ Steady state measurement under canopy (complete test after REE stable for 5 minutes) Start time_____ End time_____	
Height, Weight, BMI	Hgt_____cm. Wgt_____lbs. BMI_____kg/m^2	
Waist/Hip measurements	Waist (umbilicus)_____cm. Waist (iliac crest)_____cm. Hips_____cm.	
Bioelectric Impedance Analysis (BIA)	Resistance_____ohms Reactance_____ohms	
Baseline blood draw & hunger/satiety questionnaire	Blood sample: Questionnaire: Baseline_____ _____ By_____	
Breakfast consumed	❑Luna bar & milk ❑English muffin, cheese, apple juice Start time_____ End time_____	
Rested quietly for next 2 hours with standardized video and periodicals Blood draw and hunger/satiety questionnaire completion at 30, 60 and 120 minutes following completion of breakfast	Resting start_____ End_____ Blood samples: Questionnaire: 30 mins._____ _____ 60 mins._____ _____ 120 mins._____ _____ By_____	
Completion: • Snack prior to leaving • Record food eaten in food & PA diary • Schedule time to call & review diary entries • Confirm next testing visit date (if applicable)	Snack choice: ❑Luna Bar & H_2O ❑Slim Fast Shake & H_2O Scheduled phone call time:_____	

Signature_____ Date_____

APPENDIX E

BREAKFAST CONDITION DATA SHEET

Breakfast Testing Session Data Sheet

Subject ID:_____ **Date of Visit:_____**

Body Wt:_____lb. Ht:_____cm Age_____y

Basal Energy Expenditure*:_____ kcals x 1.3 (activity factor) = Est. daily needs:_____kcals

Est. daily needs x 0.20 = Breakfast kcal needs:_____kcals

Meal Selection: ❑ Luna Bar & milk ❑ English muffin, cheddar cheese, apple juice

Food Item	Portion Size	Kcals
Luna Bar (flavor:_____)	_____oz	
Whole milk: ❑regular ❑Lactaid	_____fl oz	
		Total:_____
English muffin	_____oz	
Cheddar cheese	_____oz	
Apple juice	_____fl oz	
		Total:_____

Breakfast start time:_____ Breakfast end time:_____

Resting start time:_____ Resting end time:_____

*Basal Energy Expenditure calculated using the Mifflin-St. Jeor formula

APPENDIX F

HUNGER AND SATIETY VAS QUESTIONNAIRE

Hunger and Satiety Visual Analogue Scales (VAS)

To be completed by staff

Date:_____ ID Number:_____ Time:_____

❑ Baseline ❑ 30 minute ❑ 60 minute ❑ 120 minute

For each question below place a single vertical slash through the line at the point that best describes how you feel right now.

Overall, how hungry do you feel?

I am not hungry at all — I have never been more hungry

_____

Overall, how satisfied do you feel?

I am completely empty — I am very satisfied

_____

Overall, how full do you feel?

Not at all full — Totally full

_____

Overall, how much do you think you could eat right now?

Nothing at all — A lot

_____

Overall, how thirsty do you feel?

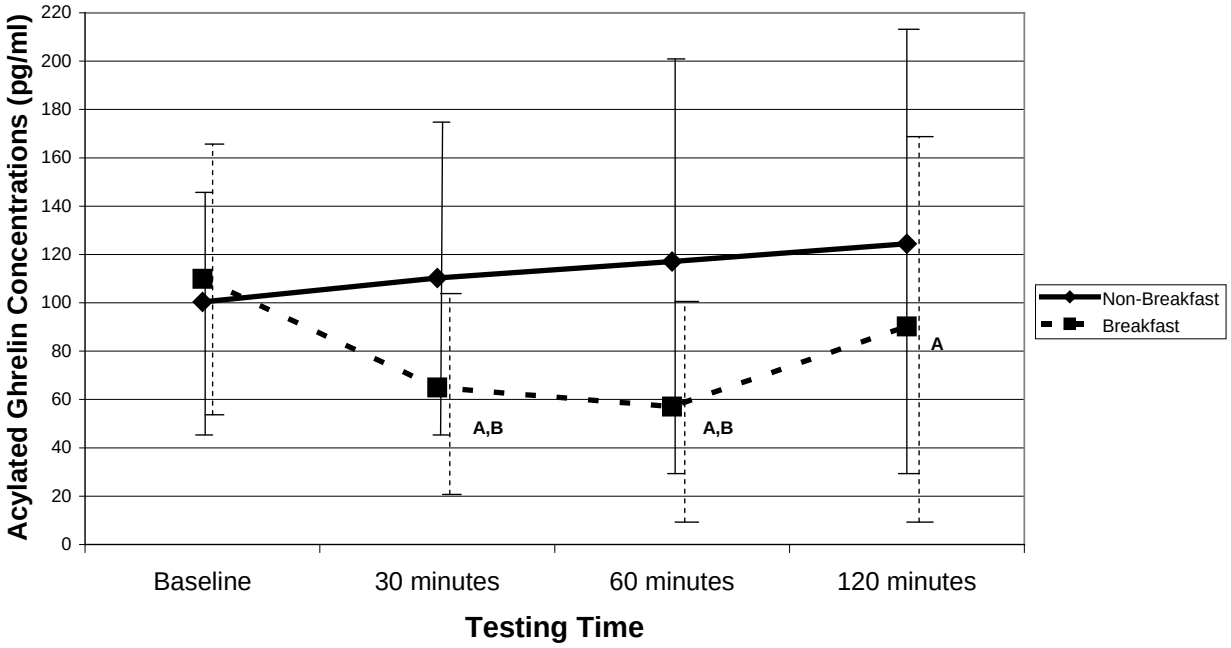
I am not thirsty at all — I have never been so thirsty

_____

APPENDIX G

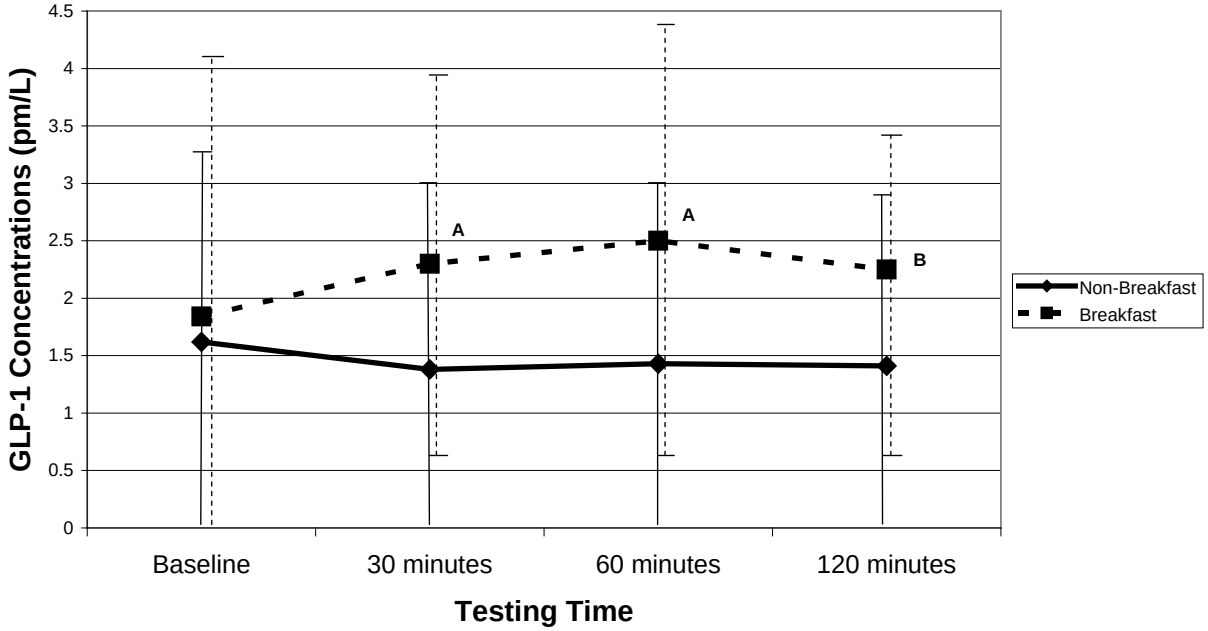
GRAPHS DEMONSTRATING CHANGES IN ACYLATED GHRELIN AND GLUCAGON-LIKE PEPTIDE 1 (GLP-1) BETWEEN BREAKFAST AND NON-BREAKFAST CONDITIONS

Changes in Acylated Ghrelin between Breakfast and Non-Breakfast Conditions (n=15)



Data presented as mean ± standard deviation
A Different than the same time point for the Non-Breakfast condition (P≤0.001)
B Different than baseline (P≤0.001)

Changes in GLP-1 between Breakfast and Non-Breakfast Conditions (n=17)

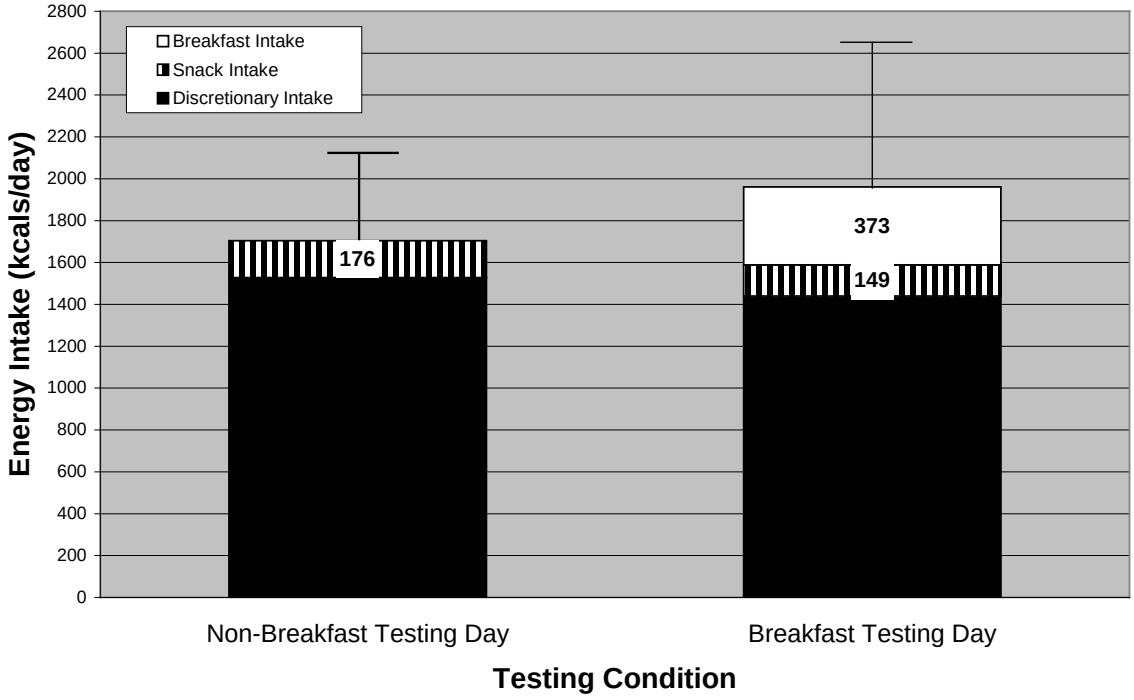


Data presented as mean ± standard deviation
A Different than the same time point for the Non-Breakfast condition (P≤0.001)
B Different than the same time point for the Non-Breakfast condition (P<0.05)

APPENDIX H

GRAPH OF DIFFERENCES IN DAILY ENERGY INTAKE BETWEEN BREAKFAST AND NON-BREAKFAST CONDITIONS

Differences in Daily Energy Intake between Breakfast and Non-Breakfast Conditions



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